Advanced Healthcare Materials

Advances in functional assemblies for regenerative medicine

--Manuscript Draft--

Manuscript Number:	adhm.201500412R1
Full Title:	Advances in functional assemblies for regenerative medicine
Article Type:	Invited Progress Report
Section/Category:	By Invitation Only: Supramolecular Assemblies for Soft Tissue Repair
Keywords:	Supramolecular; Tissue Engineering; Drug delivery; Nanotechnology; nanocomposite
Corresponding Author:	Manus Biggs National University of Ireland Galway Galway, Galway IRELAND
Additional Information:	
Question	**Response**
Please submit a plain text version of your cover letter here. **If you are submitting a revision of your manuscript, please do not overwrite your original cover letter. There is an opportunity for you to provide your responses to the reviewers later; please do not add them here**.	29th May 2015 Dear Prof. Stimson, Please find attached a copy of our manuscript entitled, “Advances in functional supramolecular assemblies for regenerative medicine”, for your consideration for inclusion in Advanced Healthcare Materials, and specifically for inclusion in the Special Issue – Supramolecular Assemblies for Soft Tissue Repair. This manuscript addresses advances in functional supramolecular materials and methodologies for engineering such. We discuss these materials in relation to tissue engineering and advances in regenerative medicine. In particular we discuss supramolecular polymer, peptide and protein arrays and novel lithographic techniques for the fabrication of three-dimensional regenerative scaffolds. We hope that you, and your referees find the revised manuscript suitable for inclusion in Advanced Healthcare Materials, and we declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We wish to confirm that there are no known conflicts of interest associated with this publication, that all sources of funding have been acknowledged and there has been no significant financial support for this work that could have influenced its outcome. Yours sincerely, _____ Dr Manus Biggs (B.Sc, M.Sc, PhD) Centre for Research in Medical Devices (CÚRAM), National University of Ireland, Galway
Corresponding Author Secondary Information:	

Corresponding Author's Institution:	National University of Ireland Galway
Corresponding Author's Secondary Institution:	
First Author:	Matteo Palma
First Author Secondary Information:	
Order of Authors:	Matteo Palma
	John Hardy
	Ghazal Tadayyon
	Maria Farsari
	Shalom Wind
	Manus Biggs
Order of Authors Secondary Information:	

Advances in functional assemblies for regenerative medicine

Dr. M. Palma[1], Dr. J.G. Hardy[2], Ms. G. Tadayyon[3], Dr. M. Farsari[4], Prof. S.J. Wind[5], Dr. M.J. Biggs[3]*

[1] Queen Mary University of London, UK

[2] Lancaster University, UK

[3] Centre for Research in Medical Devices (CURAM), National University of Ireland Galway, Ireland

[4] Institute of Electronic Structure and Laser, Crete, Greece

[5] Columbia University, New York, USA

*Corresponding Author

Centre for Research in Medical Devices (CURAM), National University of Ireland Galway, Biosciences Research Building, Newcastle Road, Dangan. Ireland

Tel: +353 (0)91 49 4328

Email: manus.biggs@nuigalway.ie

Key words

Tissue engineering; drug delivery; nanotechnology; assembly; supramolecular; functional materials

1. Introduction

The ability to produce functional assemblies of molecules and control their physical and chemical properties in the solution/solid state has found significant applications in biomedical engineering. In particular, the design and fabrication of novel biomaterials, via supramolecular chemistry approaches,[1] and innovative nano/micro fabrication strategies has facilitated the design of biocompatible materials with highly desirable properties.

Materials in which the specific intra-/inter-molecular interactions of their components drive their assembly into hierarchical (and potentially functional) assemblies abound in nature (e.g. catalytic enzymes or mechanically strong structural proteins such as mussel byssus or silks), and offer inspiration for the supramolecular engineering of materials properties. Polymers incorporating such supramolecular organization are an emerging class of novel materials because they integrate the physicochemical (and in certain cases mechanical) properties present in conventional polymeric materials with dynamic reversibility originating from the reversibility of the intra-/inter-molecular interactions at specific length scales. Consequently, the parameters that traditionally determine the properties of a polymer, such as chain length, crosslinking, chain dynamics, and chain conformation, can be reversibly adjusted in situ, leading to the development of stimuli responsive biomaterials or selectively active biochemistries. The ability to synthesise bioresponsive systems using polymer-based materials with supramolecular features has led to a surge in research interest directed towards their development as next generation biomaterials for drug delivery, medical device design and tissue engineering.

Concurrently, the industrial model for fabrication of materials is undergoing a revolution facilitated through the development of advanced formulation processes and methodologies. In particular, significant advances in regenerative scaffold formation (i.e. as an artificial extracellular matrix) are emerging from the fields of additive manufacturing, bioprinting and nanolithographic fabrication. These approaches have synergistically facilitated the development of biomaterials by enabling biomimicry on novel length scales which may enhance their physicochemical properties[2]. Current processing techniques allow the generation of materials with micron to nanoscale architecture/topography, and offer the potential to pattern bioactive molecules with high spatial resolution. Encouragingly, the combination of next-generation methodologies with medical imaging techniques is helping to drive a personalised medicine approach towards biomaterial design.

Here we review recent advances in functional assemblies for biomedical applications, with an

emphasis on novel approaches in the design of self-assembled, biomimetic and bioresponsive biomaterials for drug delivery and tissue regeneration. Advances in the use of DNA as a structural material for bioengineering are presented. Moreover, the controlled self-assembly of peptides, proteins and polysaccharides into intrinsically biocompatible soft materials is discussed in terms of their supramolecular design and different applications. We further review the use of supramolecular assemblies of polymers (biopolymers and shape memory polymers) for regenerative medicine applications. Finally, we discuss the prospects for using 2D and 3D printing technologies to fabricate biocompatible substrates and scaffolds.

2 DNA assemblies

Polynucleic acids such as DNA/RNA can self-assemble with high fidelity rendering them a powerful template for the organization and deposition of unique chemistries in high resolution at the nanometre scale. Although pristine DNA chemistries can be assembled with very high order, an area of research is emerging which focuses on supramolecular DNA assembly and exploiting DNA interactions to create highly-ordered chemical arrays. Specifically, DNA building blocks can be coupled with synthetic chemistries, polymers and nanostructures. Thus the approaches of supramolecular chemistry are augmented through the predictable and modular nature of DNA interactions.

The chemical composition of DNA along with its highly specific base pairing rules endow it with a unique programmability inspired by the genetic code of life. The notion that DNA could be programmed to assume new structural motifs was first articulated by Seeman in 1982, with the introduction of branched DNA junctions that could be assembled via the ligation of double stranded DNA (dsDNA) oligomers with sticky ends (extensions to one of the strands that could hybridize with a complementary sequence on another appropriately designed dsDNA oligomer,[3] **Figure 1A and 1B**. Based on this approach, a variety of structures have been synthesized, including lattices,[4] cubes[5] and closed polyhedral.[6] 2D crystals of double crossover (DX) DNA have also been formed by sticky end ligation to form DNA tiles,[7] which can assemble into periodic 2D lattices comprising thousands of constituent tiles. Complex, aperiodic assemblies of DNA tiles can be formed by using a preassembled input DNA strand that encodes the required pattern information, around which other oligonucleotides assemble into specified tiles.[8] Aperiodic arrays can also be assembled algorithmically starting from a well-designed tile seed[9] in a form of computational assembly. In addition to 2D arrays, 3D DNA wireframe nanostructures,[10] as well as 3D DNA crystals,[11] have also been synthesized by sticky end ligation, with some designs reaching

macroscopic dimensions.[12] **Figure 1C and 1D** shows several representative examples of DNA structural assemblies formed by sticky end cohesion.

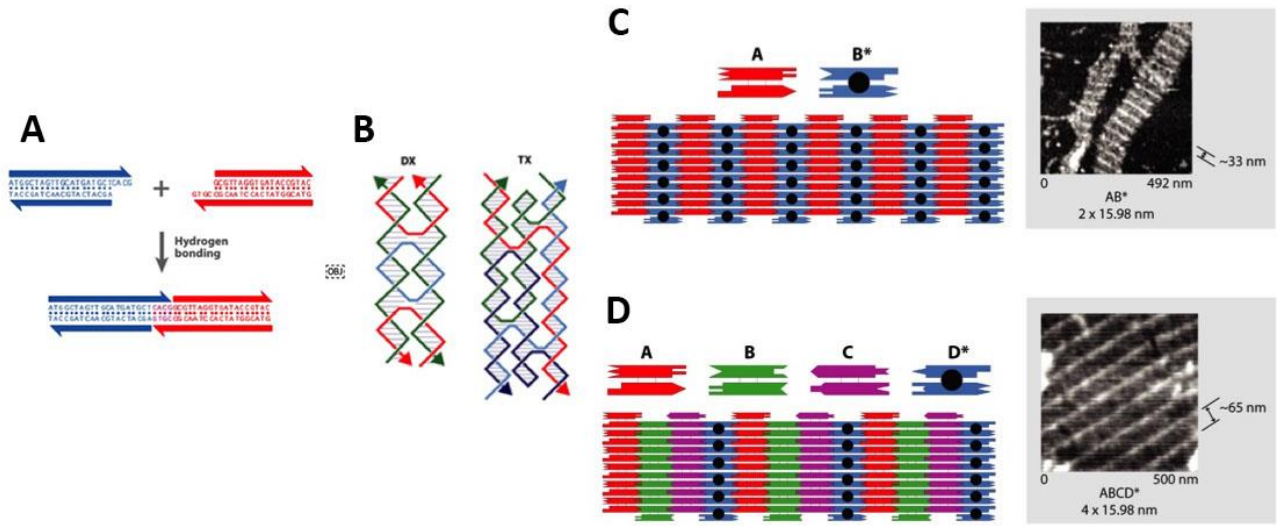

Figure 1. Programming DNA to assume new structural motifs. (A) Sticky end cohesion and ligation. (B) Double and triple crossover DNA structural motifs. (C,D) Two-dimensional arrays of DNA nanostructures formed by sticky end cohesion with associated AFM images of synthesized structures. Modified from N. C. Seeman and reproduced with permission of the publisher.[13]

Even more complex DNA nanostructures became possible with the advent of DNA origami.[14] Formation of DNA origami involves the intricate folding (**Figure 2A**) of a single scaffold strand of viral DNA by approximately 200 short strands, called "staples," that bind together specific sequences along the viral strands in a pre-programmed manner, resulting in the creation of the desired shapes (**Figure 2B**). DNA origami surpasses the tiles mentioned above in size and scale. Whereas the building blocks of the tiles contain about 100-500 nucleotide pairs, the DNA origami scaffold consists of about 8000 nucleotides (the staples are approximately 40 bases) [15] The result is that a single origami structure is approximately 100 nm on a side. Of course, for many applications, larger structures are required, and DNA origami can be made to self-assemble into arrays using sticky ends at selected sites on the edges of the origami. Linear,[16] crossing[17] and more arbitrary 2D networks[18] are possible using different hierarchical organization schemes.

3D origami structures are also possible (**Figure 2C**), and are emerging as promising tool for both materials science and biological applications.[19] Hollow cubes or cages, formed by interconnecting multiple origami sheets using staple strands at their edges[20] can be opened or closed using an appropriate DNA "key". Solid origami structures are formed by stacking double helix cylinders that comprise scaffold and staple strands into multiple layers.[21] Notably, these structures are formed in a one-pot reaction, as is planar origami. Different solid shapes (e.g., bricks, rods, crosses, nuts, etc.) can be built by modifying the size of the cylinders and the staples that connect them.[21]

Larger structures can be achieved by bridging between the solid building blocks using additional staple strands. Careful design of the cylinders can build a small degree of strain between adjacent layers, resulting in curved, solid origami structures with complex designs.[22, 23]

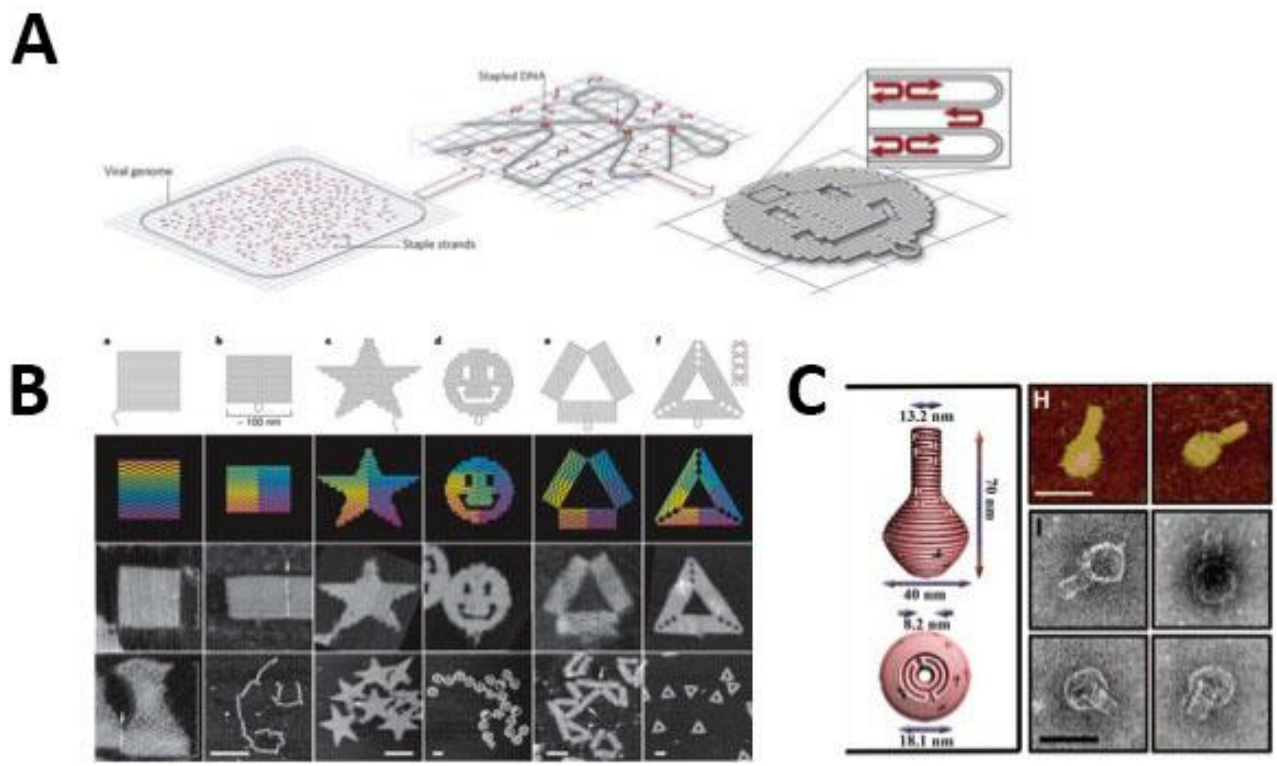

Figure 2 Supramolecular DNA arrays formulated thought the DNA origami process. (A) DNA origami concept showing the stapling together of a viral strand of DNA with short oligomers (B) Gallery of origami shapes (C) Curved 3D DNA origami nanostructures. Modified from Seeman et al., Rothemund et al. and Han et al. and reproduced with permission of the publisher[14, 23, 24]

The notion that DNA-based structures could be used as scaffolds for the assembly of biological[25] and non-biological materials was first articulated by Seeman in 1990.[26] 1D[27] and 2D[28] periodic arrays of inorganic materials, such as Au nanoparticles and semiconducting quantum dots[29], have been created using DX tiles. 2D DNA origami scaffolds have been the subject of intense study for this type of assembly, with demonstrations of assembly of homogeneous metallic nanoparticles,[30] quantum dots[31] and even carbon nanotubes[32]. The keys to this sort of decoration of the origami with non-biological materials are (a) the functionalization of the material with a biomolecule and (b) modification of the staple strands on the origami with a complementary linker. This has recently been exploited to assemble heterogeneous nanomaterials, including different size metallic nanoparticles and different aptamers which have been placed on the same origami scaffold using this approach with a spatial resolution of approximately 6 nm.[33]

Shortly after the first report of 2D DNA crystals,[7] such structures were further used to create arrays of DNA and protein molecules[34]. Interestingly, Koyfman et al have demonstrated the specific attachment of periodic 2D DNA arrays to cells using two different methods involving biotin-streptavidin and specific antibody-cell surface[35]. Via precise DNA origami design and through their controlled interaction with the cell surface, this approach paves the way to the engineering of cell/surface and cell/cell networks into microtissuses.

Self-assembly of DNA has recently been identified as a powerful approach for targeted intracellular delivery of therapeutic payloads through the formulation of nanoparticulate carriers.[36, 37] Interestingly, DNA nanostructures can readily pass through the membrane barrier[38] and survive within cells from 24 up to 60 hours[39]. 1D DNA nanostructures exhibit cellular uptake [40], with rolling circle amplification-templated DNA nanotubes showing increased stability and the capability of entering human cervical cancer[41]. Cell internalization has also been quantified for 2D DNA origami[42]. Moreover, cell entry pathways of 3D DNA (tetrahedral) nanostructures have notably been investigated[43] and modulated via single-particle tracking.[44]. Additionally the stability of DNA nanostructures in tissue culture has been recently thoroughly investigated[45].

A recent study by Sellner *et al.* reported on the DNA tile-assembly method to synthesise DNA-based nanotubes as carrier systems for cytosine-phosphate-guanine (CpG) sequences *in vivo*. Local microinjection of DNA nanoconstructs in intact muscle tissue of healthy mice tissue were observed to accumulate intracellularly in resident macrophages. The inclusion of CpG sequences along the nanotube chemistry induced a strongly elevated immune response, particularly the recruitment of leukocytes from postcapillary venules to the tissue and nuclear translocation of p65, a subunit of the NF-kB transcription factor complex, which is commonly used as an indicator of NF-kB activation.[46] 3D DNA nanostructures have also been employed for intracellular delivery, allowing the transport of immunostimulatory oligonucleotides via the use of CpG functionalised DNA tetrahedra[47]. These functional nanostructures have shown the ability to enter macrophage-like RAW264.7 cells without the aid of transfection agents. Following their cellular uptake, downstream pathways were activated thanks to the presence of the CpG motifs, inducing immunostimulatory effects [47].

Employing a different apporach, Li *et al.* explored Y-shaped monomers with three sticky ends, Y-shaped monomers with one sticky end, and DNA linkers with two sticky ends as building blocks for size-controllable and stimuli-responsive DNA nanohydrogels and investigated these DNA hydrogels as effective targeted gene delivery vectors.[48] The authors concluded that these hydrogels were

effective for targeted and stimuli-responsive gene therapy, noting that cancer therapy strongly inhibited cell proliferation and migration in target A549 cells.

DNA self-assembly has also been employed to develop a nuclear-uptake nanodrug system carried by a cell-targeted near-infrared (NIR)-responsive nanotruck for drug-resistant cancer therapy. Via DNA hybridization, small drug-loaded gold nanoparticles were self-assembled on the surface of a silver-gold nanorod which was also modified with a cell type-specific internalizing aptamer. By using this nanodrug delivery system, anticancer drugs were efficiently accumulated in the nuclei to effectively kill cancer cells.[37]

Aside from delivery [38, 49] complementary to the efforts to assemble functional materials on DNA origami is work aimed at controlling the placement of the origami on surfaces.[50] This is driven by the fact that most of the assembly on origami to date is oriented toward applications in nanoelectronics and plasmonics. Lipid-bilayer-anchored DNA origami structures can be assembled into prescribed superstructures in a programmed manner. The reported DNA-based artificial system can mimic the dynamic assembly of membrane-associated protein clusters that play an essential role in deformation of cellular membranes.[51]

By and large, the spatial targeting and multivalent properties[33] of DNA nanostructures are unrivaled by existing techniques. This makes them ideal nanoplatforms for biological applications[25]. DNA nanostructures have indeed been employed to transport molecular payloads to cells[52], for cancer-drug delivery[53], to map pH gradients along different cellular entry pathways[54], as synthetic vaccine platforms[55], to deliver siRNAs into cells to silence target genes[56], as intracellular logic sensors[57], and as biological “computing platforms” in living animals[58].

3. Poly(amino acid) assemblies

3.1. Peptide assemblies

The design and synthesis of peptides that self-assemble controllably into ordered nanostructures is a powerful approach for the development of soft biomaterials[59]. Moieties capable of mimicking the natural ECM environment can be employed both in fundamental studies of cell-matrix interactions, and in the development of biomimetic materials for regenerative medicine and tissue engineering that have prospects for clinical translation[60].

In this context, peptides are ideal building blocks because of their intrinsic biocompatibility and the multitude of strategies that can be adopted to fabricate novel materials via self-assembly.[61] Various intermolecular interactions have been employed in the design of peptide-based self-assembling materials, including hydrogen bonding, electrostatic interactions, as well as hydrophobic interactions (between aliphatic residues) and π-π stacking (between aromatic residues).[62] Peptide materials have been designed to form supramolecular nanostructures presenting biochemical and physicochemical cues that control cell behaviour.[63, 64, 65] The strategies employed have ranged from biologically inspired ones (e.g. α-helix coils, β-sheets, collagen), to novel approaches (cyclic peptides and amphiphiles).[66] Different shapes, such as micelles, vesicles and nanofibres, have been obtained.[67] Moreover, the 3D spatial distribution of chemical moieties has been controlled by changing the peptide conformation to more accurately mimic the natural biochemical cues of the ECM-cell interface.[68, 69]

Fibrous supramolecular nanostructures formed via peptide self-assembly have been designed to display cell-binding epitopes. β-sheet fibril scaffolds are typically obtained by alternating polar/non-polar amino acid sequences, while two antiparallel β-strands can be joined by a loop to form β-hairpins. Additionally, β-helices can associate with each other to form coiled-coils. Triple helical assembly that mimic collagen can also be obtained with Pro-Hyp-Gly peptides units.[64] Interestingly, De Santis *et al.* have designed a symmetrical sequence template comprising two generic α-helical modules, N- and C-terminal, with the same number of heptads (structural motifs that consist of a repeating pattern of seven amino acids). They studied the formation of supramolecular fibres varying the total number of heptads, and promoted staggered coiled-coil assembly via oppositely charged heptads.[70] Different assembly patterns resulted from the synergistic interplay between peptide length, net charge and folding, and supramolecular cooperativity.[70] Moreover, the same research group has reported a self-assembling peptide gel that supports mammalian cell proliferation and resists bacterial colonization. These fibrous networks proved to be attractive biomimetic architectural models for different extracellular matrices.[71]

Nanofibres formed via self-assembly of peptide amphiphiles (PAs), have also generated great interest in the field of regenerative medicine.[69] PAs tend to contain alkyl tails covalently attached to the end of a peptide chain, encouraging their self-assembly into anisotropic nanostructures that can chemically and mechanically interact with cells. PA nanofibres presenting a neurite-promoting laminin epitope IKVAV were shown to induce selective differentiation of neural progenitor cells.[72]

The direct injection of these peptide amphiphile solutions into rat spinal cords was further shown to trigger the localized self-assembly of the aforementioned 3D scaffold and promote axon elongation after injury.[73] More recently, Mammadov *et al.* developed PA nanofibres containing laminin-derived peptide signals along with a heparan-sulfate-mimicking group.[74] These scaffolds significantly promoted neurite outgrowth by PC-12 cells and were shown to be effective even in the presence of inhibitory components of the central nervous system.

Interestingly, self-assembling peptides carrying a di-glycine linker and the functional motifs SKPPGTSS, PFSSTKT and RGD, were recently reported to act as nanofibre-based 3D tissue culturing systems for neural cells by Koutsopoulos and Zhang.[75] The authors of this work showed that neural stem cells present marked differentiation into projection neurons, astrocytes and oligodendrocytes when encapsulated in the aforementioned hydrogel matrices. Moreover, the long-term (up to 5 months) culturing in serum-free medium achievable with this system, can allow for more realistic biological studies of neural cells in a biomimetic 3D environment (**Figure 3**).

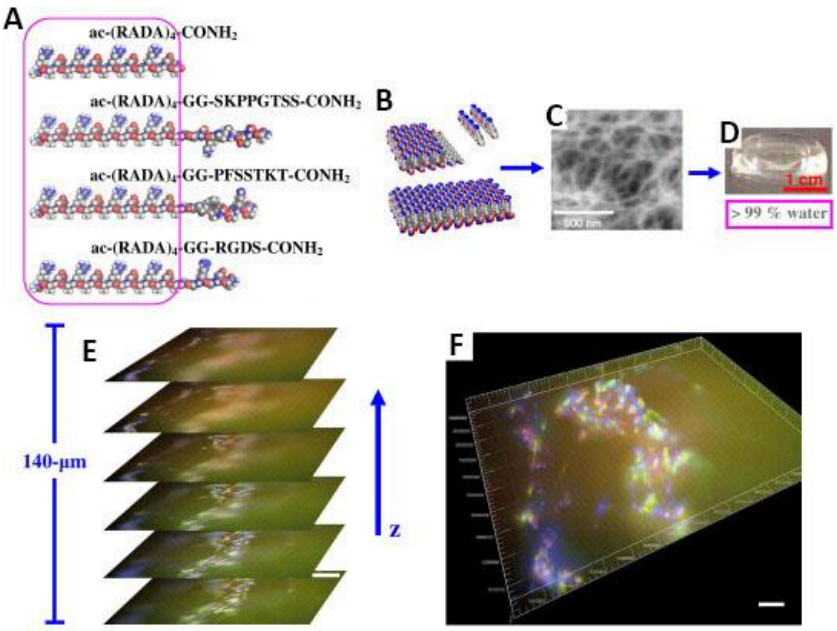

Figure 3 Molecular models of a self-assembling peptide ac-$(RADA)_4$-$CONH_2$ and of a modified self-assembling peptide carrying a di-glycine linker and the functional motifs SKPPGTSS, PFSSTKT and RGDS. Color code: carbon, grey; oxygen, red; nitrogen, blue; hydrogen, white. (B) Molecular model of the self-assembling peptide and of the nanofiber formed upon assembly of the peptide monomers. (C) SEM picture of the peptide nanofibers inside the hydrogel. (D) Picture of the peptide hydrogel. (E-F) Microscopy image volume representation of neural cells encapsulated in the peptide hydrogel. Images were aquiredf at 2 week culture in which nestin(+) neural progenitors, cells (green) and Tuj1(+) neurons (red) appear at different z-planes; Scale bar is 200 µm. Modified from Koutsopoulos et al and reproduced with permission of the publisher.[75]

Ionic self-complementary peptides and PAs have also been developed as β-sheet forming systems that assemble into nanofibres and 3D scaffolds for nerve repair.[76] Very recently, Li *et al.* have shown that aligned RGDS-PA gels are able to satisfy the requirements of mimicry of native nerve

ultrastructure and bioactivity.[77] Schwann cell proliferation, attachment, and alignment were demonstrated employing the aforementioned biomaterial. Remarkably, in vivo testing revealed the recovery of motor and sensory function in animals treated with conduit/PA constructs.[77] A different study by Angeloni *et al.* showed that sonic hedgehog (SHH) treatment of cavernous nerves via aligned PAs nano-fibres significantly improves erectile function.[78] The use of (C16)-V2A2E2-(NH2) PA monodomain noodle gels was shown to promote nerve regeneration and suppress penile apoptosis.[78] This study demonstrated the important role of SHH in the regeneration of CN in prostatectomy and diabetic patients, potentially paving the way to important clinical applications.

Recent reviews have also discussed the application of peptide nanofibres in bone regeneration and biomineralization.[64, 65] Notably a 3D self-assembling leucine zipper (LZ) hydrogel that was synthesized and functionalized with RGD domains. In vivo implantation of the LZ scaffolds in a mouse model showed them to be relatively non-immunogenic (i.e., absence of a foreign body reaction to the scaffold), while experiments with human marrow showed the biological property of the hydrogel to promote cell attachment/proliferation and its ability to support neovascularization.[79]

Additionally, self-assembling peptide scaffolds have been successfully employed for cartilage tissue engineering. Peptide-based nanofiber networks highly resembling natural extracellular matrixes have been shown the ability to regenerate cartilage tissue both in vitro and in vivo.[80] Peptide amphiphiles designed to form nanofibres and display a high density of binding epitopes to transforming growth factor β-1 (TGFβ-1), have been employed for articular cartilage regeneration.[81] In vitro experiments indicated that these materials promote the chondrogenic differentiation of human mesenchymal stem cells (MSCs). Additionally, these epitope-modified supramolecular nanofibres enhanced the regenerative potential of microfracture-treated chondral defects in a rabbit model.

Angiogenesis, the process of new blood vessel generation, plays an important role in tissue growth and regeneration. Peptide-based assemblies have been employed to enhance or inhibit this process.[64] PA-based nanofilaments have been designed to display on their surfaces a VEGF-mimetic peptide, because VEGF is one of the most potent angiogenic signaling proteins.[82] Proangiogenic behaviour in endothelial cells was demonstrated in vitro, while in vivo experiments showed that the nanofibres increased tissue perfusion, functional recovery, limb salvage, and treadmill endurance. In a more recent study, the same research group has discussed the synthesis

and the anti-angiogenic activity, both in vitro and in vivo, of supramolecular nanostructures formed via the self-assembly of small PAs containing the G-helix motif of maspin.[83] In vitro cell assays demonstrated that these short nanofibres inhibit endothelial cell motility via G-helix mediated pathways, and block tubulogenesis at sub-micromolar concentrations. In vivo studies further showed evidence of the nanostructures' effectiveness at inhibiting angiogenesis in the chicken embryo chorioallantois. Notably, Kumar *et al.* have reported multidomain peptide sequences (MDPs) conjugated with a VEGF mimic to promote angiogenesis. The MDPs consisted of terminally charged residues that flank alternating hydrophilic and hydrophobic residues and associate into bilayers of antiparallel β-sheets. Kumar *et al.* have synthesized MDPs with KKSLSLSLSLSLSLSLKK as the base peptide sequence, modified with a VEGF mimic. Nanofibrous hydrogels of these peptides were shown to be cytocompatible, and were delivered by simple syringe injection demonstrating excellent tissue integration. The hydrogels were rapidly infiltrated by hematopoietic and mesenchymal cells forming a robust vascular network.[84]

Anti-inflammatory peptide amphiphiles have also been used in tissue regeneration, and exhibited potent angiogenic responses, limited tissue collagen accumulation, and the modulation of macrophage response in regenerated bladder tissue.[85] Additionally, heparin-binding PAs (HBPA) have been designed to release growth factors for both angiogenesis and cardiovascular disease.[86] Heparin is a natural biopolymer known to interact with different growth factors. Self-assembling peptide nanofibres presenting heparin were shown to bind paracrine factors. Moreover, significant preservation of haemodynamic function was observed when these nanomaterials were injected into the heart following coronary artery ligation in a mouse ischaemia-reperfusion model of acute myocardial infarction.[87]

Interestingly, cell fate can be controlled via the proper design of self-assembling peptide-based nanostructures. Kuang *et al.* have demonstrated that hydrogels/nanonets of self-assembled aromatic tripeptide amphiphiles can selectively form around the pericellular space of cancer cells that overexpress phosphatases and thereby induce apoptosis of the cancer cells.[88] Differently, Stupp and *et al.* have systematically studied the influence of hydrogen bonding, hydrophobic domains and charge of PA materials on their interactions with cells.[89] They demonstrated that cell viability is affected by intermolecular interactions: the disruption of the cell membrane can be induced by the interaction of less cohesive assemblies with the lipid membrane and materials that induce cell death can create a barrier to cell migration in 3D cultures.[89]

In order to combine the intrinsic biocompatibility and nanoscale control of peptide assemblies with the stability and processability of synthetic polymers, different studies have exploited co-assembly strategies between polymers and peptides for scaffold fabrication in tissue engineering. Indeed, peptide-polymer conjugates have been co-electrospun with a host polymer to fabricate peptide gradient scaffolds.[90] The peptide-polymer conjugate system specifically and dynamically bind glycosaminoglycans secreted by cells. Employing a different approach, Capito *et al.* have reported on the formation of macroscopic sacs membranes used to encapsulate cells. These sacs were obtained via the self-assembly of a charged megadalton polymer and peptide amphililes.[91] Various membranes were further fabricated via the selective assembly of hyaluronic acid with positively charged PA containing anti-cancer PAs bearing a $(KLAKLAK)_2$ peptide sequence. In this way ad hoc designed membranes were able to be used as reservoirs for sustained release of cytotoxicity (upon enzymatic degradation).[92] In a similar way, Mendes *et al.* used a positively charged multidomain peptide, with and without the arginine-glycine-aspartic acid-serine (RGDS) cell-adhesive peptide sequence, and a high molecular weight negatively charged biopolymer, to assemble bioactive membranes.[93] A photolithography patterning process was then used for the fabrication of membranes displaying posts, holes, channels and pores ranging from 10 to 20 µm. Cell adhesion, spreading, and morphology were reported to be significantly affected by the surface topographical patterns and the different concentrations of RGDS of the membranes.[93]

In this context, a more recent study has further highlighted the ability of modulating certain cellular activities through matrix engineering. Ferreira *et al.* have indeed designed a PA able to self-assemble with hyaluronan into membranes containing a proteolytic domain sensitive to matrix metalloproteinase-1.[94] This study suggests that membranes that include the matrix metalloproteinase-1 cleavable sequence stimulate protease secretion, leading to cell-mediated degradation processes.[94]

3.2. Protein assemblies

Proteins have evolved in biology for task-specific roles (catalysis, energy transfer, structure). Processes such as catalysis and energy transfer are commonly undertaken by proteins with well-defined structures in specific locations in biological pathways, whereas the proteins responsible for structural roles are either structures formed from well-defined numbers of individual protein building blocks (e.g. viral capsids) or assemblies of ill-defined numbers of individual protein building blocks (e.g. cytoskeletal actin). Proteins can be isolated from a variety of sources, including mammals (e.g. collagens, elastins, fibronectins, keratins and laminins), non-mammals

(e.g. mussel byssus, silkworm/spider silks), plants (e.g. soy or zein) and recombinamers (genetically engineered recombinant proteins typically produced by fermentation in bacteria or yeast), and are widely used in drug delivery and tissue engineering.[95]

Researchers have made incredible progress in the use of viral capsids as self-assembling templates for new materials with potential for application in nanotechnology (e.g. light harvesting, photocatalysis, biomedicine) [96] . Virus-based or virus-inspired assemblies represent drug and nucleic acid delivery devices in which the biodistribution of the therapeutic agent can be controlled by ligand-cell receptor interactions, which may markedly enhance the effectiveness of the drug (and ideally reduce side effects associated with the toxicity of the therapeutic), encouraging the development of virus-like protein assemblies/cages exhibiting viral functions such as cell recognition/penetration and compartment-aimed payload delivery. Consequently, virus-based or virus-inspired assemblies are a hot topic in the field of protein engineering drug delivery,[97, 98, 99] or indeed tissue engineering,[100] and various approaches to their safe manufacture and use are under investigation.[101]

The natural role of viruses as vectors for DNA and RNA delivery inspired their use for the delivery of therapeutic DNA and RNA payloads in the field of gene therapy, now a multimillion dollar industry. Gene therapy was first used commercially in China for the treatment of certain cancers with Gendicine; thereafter, gene therapies were developed in Russia for the treatment of peripheral artery disease with Neovasculgen®, and the treatment of lipoprotein lipase deficiency with Glybera in Europe. Gene therapies are currently being investigated for a variety of conditions, including haemophilia, lymphocytic leukemia, multiple myeloma and Parkinson's disease, and we expect to see many more clinically translated gene therapies in coming years.

It is noteworthy that the viral payload need not necessarily be DNA or RNA, and the payload could instead be a low [98, 99] or high molecular weight[102] therapeutic. Indeed, the delivery of low molecular weight therapeutics such as porphyrins (capable of generating singlet oxygen upon illumination) facilitates the application of such nanoscale assemblies for photodynamic therapy for the treatment of cancers or pathogens[103]. Likewise, it is possible to deliver drugs to specific tissues (e.g. liver[104], colon[105]) including tissues that are typically challenging to deliver drugs to (e.g. across the blood-brain barrier [106]), highlighting their prospects for use as theranostic agents (i.e. agents capable of diagnosis of a condition and its treatment)[107] [108].

Furthermore, viruses and virus-like assemblies can be repurposed for tissue engineering applications. Indeed, two-and three-dimensional scaffolds based on such assemblies can be

employed as an artificial ECM capable of regulating cellular functions as reviewed by Zhao and *et al*.[100] Such scaffolds are particularly interesting as viruses such as Tobacco mosaic virus has been shown to have low immunogenicity towards mammals (e.g. mice) [109].

Biomaterials based on other proteins are incredibly popular biomaterials; their popularity stems from their ease of processing into various materials morphologies (e.g. fibers, films, foams, gels or particulates), the structural diversity of the proteins available (both from natural and recombinant sources) which yields materials with highly tunable chemical, mechanical and topographical properties. A comprehensive overview is beyond the scope of this review as the literature is even vaster than that for self-assembling peptides, therefore we direct the reader towards a selection of reviews [[110, 111]. Clearly, when contemplating preparing non-viral protein-based biomaterials the use of proteins isolated from the extracellular matrix (collagen, elastin, fibronectin, laminin) is popular, in part because we may be able to use a patient's own ECM-derived proteins to make such materials which is particularly important in patients with weak immune systems. Consequently, there are abundant reports of extracellular matrix protein-based biomaterials either as isolated components, or as mixtures isolated after decellularization processes[112]. There are also interesting reports of ECM-mimetic recombinamers which yield proteins with improved solubilities than their natural counterparts (e.g. elastin-mimetic proteins) [113]; and indeed non-mammalian proteins Soy[114], Zein[115], Mussel byssus[116], or silks from various species used for drug delivery[117] or tissue scaffolds (e.g. for invertebral discs[118] or bones[119]).

4. Polysaccharide assemblies

The abundant supply of natural polysaccharides such as alginate, cellulose, chitin or hyaluronic acid (mammalian ECM), and low/no immunogenic response to the polymers have made them popular components in drug delivery devices and tissue scaffolds.[120]

Alginates are isolated from the cell walls of algae and are widely used components of bulk hydrogels.[121] Doxorubicin functionalized alginates have been encapsulated inside PA nanofibers functionalized for targeting the folate receptor, and such supramolecular assemblies were shown to display 6-fold higher cytotoxicity against MDA-MB-231 breast cancer cells compared to those without the targeting groups.[122] In-situ gelation of suspensions of MSCs in alginate triggered by Ca^{2+} ions has been reported to produce injectable hydrogels that enable chondrogenic differentiation of the MSCs in vitro, and when implanted in nude mice the hydrogels facilitated cartilage tissue regeneration.[123]

While cellulose is incredibly important for building (wood) and printing (paper) industries its poor

solubility has made it challenging to work with, however the isolation of nanocellulose crystals (which are hierarchically ordered fibrillar assemblies of cellulose) has invigorated research in its application in biomedicine.[124] Supramolecular assemblies of nanocellulose fibrils decorated with β-cyclodextrins with pluronic polymers threaded through them were shown to form bulk hydrogels suitable for the delivery of doxorubicin.[125] Bulk hydrogels composed of nanocellulose fibrils embedded in gelatin-rich in-situ crosslinking gels were shown to induce the differentiation of HepaRG progenitor cells to organotypic 3D spheroids with bile duct compartments in the core that expressed hepatocyte markers, metabolic activity and vectorial molecular transport towards the bile duct compartment.[126]

Chitin is isolated from the cell walls of fungi and animal exoskeletons, and once deacetylated yields cationic chitosan.[127] Supramolecular self-assembled nanocomplexes of oleyl-conjugated trimethyl chitosan, poly(γ-(4-((2-(piperidin-1-yl)ethyl)aminomethyl)benzyl-L-glutamate), oleyl-PEG-mannose (OPM), and plasmid DNA encoding luciferase were capable of mannose receptor-mediated endocytosis and permeable to the cellular and endosomal membranes of HepG-2 cells, thereby enabling non-viral gene delivery that outperforms commercial transfection reagents, including LPF2000, PEI, and jetPEI, by up to 2 orders of magnitude.[128] With a view towards engineering the soft component of bones, supramolecular gels formed from polyelectrolyte complexes of chitosan and ulvan were shown to be suitable for osteoblasts culture in vitro.[129] The removal of a 3D printed sacrificial template embedded in hydrogen bond crosslinked assemblies of nanochitosan fibrils yielded porous hydrogels (in which the pore structure was dictated by the structure of the sacrificial template) that were coated with gelatin and calcium phosphate and shown to support the differentiation of human MSCs towards osteogenic outcomes.[130]

Hyaluronic acid is a natural non-sulfated glycosoaminoglycan that plays important roles in cell-cell interactions, cell adhesion and migration, and binds to the cell-surface glycoprotein CD44. CD44 is itself involved in a multitude of biological processes, is overexpressed in many tumours, and involved in cancer metastasis. This renders drug loaded supramolecular assemblies of hyaluronic acid interesting vehicles for cancer cell-targeted drug delivery;[131] for example decoration of hyaluronic acid with hydrophobic cholesterol moieties encouraged their self-assembly into micellar structures capable of delivering complexes of 2b RNA-binding protein and siRNA to murine melanoma (B16F10) cells and suppress the activity of the target RFP gene.[132] It would also be possible to fill the cavity left after excision of a tumour with a supramolecular hydrogel formed from mixtures of hyaluronic acid derivatives displaying curcurburitils and polyamine-decorated

hyaluronic acid derivatives. Such gels were able to support the growth and proliferation of NIH3T3 mouse fibroblast cells, and were shown to be both rapidly integrated with no noticeable immune response after 7 days of implantation in nude mice.[133] Exciting subsequent studies incorporating engineered MSCs producing enhanced green fluorescence protein demonstrated that the MSCs remain alive in the gels and emit the fluorescence when implanted in mice for more than 60 days. Interestingly, the long-term expression of mutant interleukin-12 by the engineered MSCs within the supramolecular hydrogels results in effective inhibition of tumour growth with a significantly enhanced survival rate.[134] Moreover, these hydrogels also allow the differentiation of hMSCs to be temporally controlled by changing the release profiles of transforming growth factor-β3 and/or dexamethasone from the hydrolyzable dexamethasone displaying curcurburitil derivative (Dexa-CB[6]), and have potential for cartilage regeneration (**Figure 4**).[135]

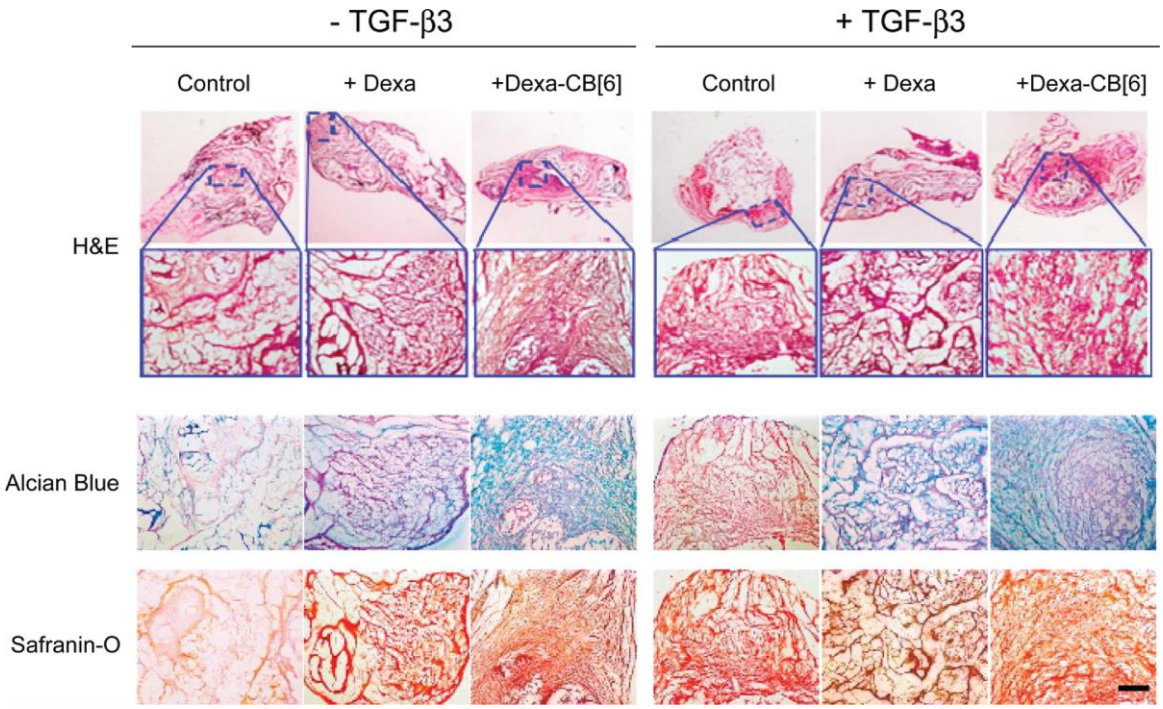

Figure 4. Histological analysis of neocartilage formation in vivo by the differentiation of hMSCs in monoCB[6]/DAH-HA hydrogels. Gels were prepared without and with free dexamethasone or Dexa-CB[6] in the absence and presence of TGF-β3 and analysis performed via staining with H&E, Alcian blue, and Safranin-O at 4 weeks. Modified from Jung et al. and reproduced with permission of the publisher.[135]

5. Synthetic polymer assemblies

5.1. Drug delivery and tissue scaffolds

Monodisperse biopolymers such as DNA and proteins have inspired the development of synthetic polymers that are monodisperse (e.g. dendrimers)[98] or effectively monodisperse (e.g. block copolymers prepared via living polymerizations).[136] Such polymers can hierarchically assemble

into well-defined polymer assemblies in direct analogy to the biopolymers that inspired their creation.[137, 138] Well-defined polymer-based assemblies of this nature are relatively new, and the development for biomedical applications has focused primarily on drug delivery systems.[138, 139] However, we believe that functional supramolecular assemblies of polymers show incredible promise for both drug delivery[140] and tissue engineering[141] as we highlight below. Some systems self-assemble because of their surfactant-like properties, whereas others require the addition of one or more components to induce self-assembly, and cyclodextrins (which form inclusion complexes with the other component(s)) are a popular means of achieving this.[142]

Polyaspartic acid residues are found in natural calcium-based biominerals,[143, 144] and polyaspartic acid-base biomaterials biomineralized with calcium phosphate have been shown to support the adhesion and proliferation of MSCs, and such materials may find application in bone tissue engineering.[143] Supramolecular assemblies of β-cyclodextrin functionalized poly(aspartic acid) derivatives with adamantane functionalized RGD peptides and adamantane functionalized camptothecin, and in vitro studies showed them to be potent delivery vehicles for camptothecin to COS 7 and HeLa cells.[145] Supramolecular vesicular aggregates composed of folate decorated poly(aspartic acid) derivatives (polyaspartyl-hydrazide copolymers) that were capable of delivering low molecular weight gemcitabine to cancer cell lines, with a significantly higher uptake by MCF-7 cells which over-express the folate receptor than BxPC-3 cells which do not over-express this receptor; their pharmacokinetics showed that they were removed from the circulatory system at a slower rate than the native drug, and prolonged gemcitabine plasma concentration was observed for up to 16 hours.[146] Likewise, supramolecular assemblies based on the FDA approved polycaprolactone[147] have been investigated for their potential in drug delivery; and supramolecular hydrogels prepared from α-cyclodextrin-based inclusion complexes with diblock copolymers (polycaprolactone-co-polyethyleneglycol) grafted with chitooligosaccharides were capable of sustained release of a model high molecular weight drug (a protein, bovine serum albumin).[148] Further, hydrogels composed of electroactive α-cyclodextrin complexes of polyethyleneglycol derivatives terminated with electroactive aniline oligomers were shown to facilitate the electrical stimulation (a square wave, frequency of 100 Hz, 50% duty cycle, and electrical potential of 0.5 V were applied for 0.5 hours every day) of rat cardiomyoblast (HC92) cells residing therein which promoted their proliferation relative to non-stimulated controls.[149]

Meijer and *et al.* have produced some of the most exciting work in this field, and developing supramolecular materials based on polymers displaying quadruple hydrogen-bonding ureido-

pyrimidinone (UPy) moieties.[150] Indeed, oligocaprolactones terminated with UPy moieties were shown to be processable into a variety of biomedically relevant materials morphologies including films, fibers, and 3D printed foams.[150] These materials supported the adhesion and proliferation of fibroblasts in vitro, and when implanted in rats, certain formulations showed evidence of vascularization after 5 days, with relatively minimal immunogenicity.[150, 151] An interesting study of supramolecular polymers formed from poly(caprolactone) derivatives terminated with quadruple hydrogen-bonding ureido-pyrimidinone moieties and hydroxyapatite nanoparticles functionalized with ureido-pyrimidinone moieties, reported their ability to support the adhesion and proliferation of MSCs, with a view to their use as bone tissue scaffolds.[152] Moreover, hydrogels formed from ureidopyrimidinone (UPy) terminated poly(ethylene glyocol) have been shown to be effective drug delivery vehicles, enabling the delivery of protein drugs to kidneys with incredibly low inflammation (14 to 23 times less endotoxin than the value permitted by the FDA);[153] and analogous gels enabled the delivery of growth factors to kidneys (**Figure 5**).[154]

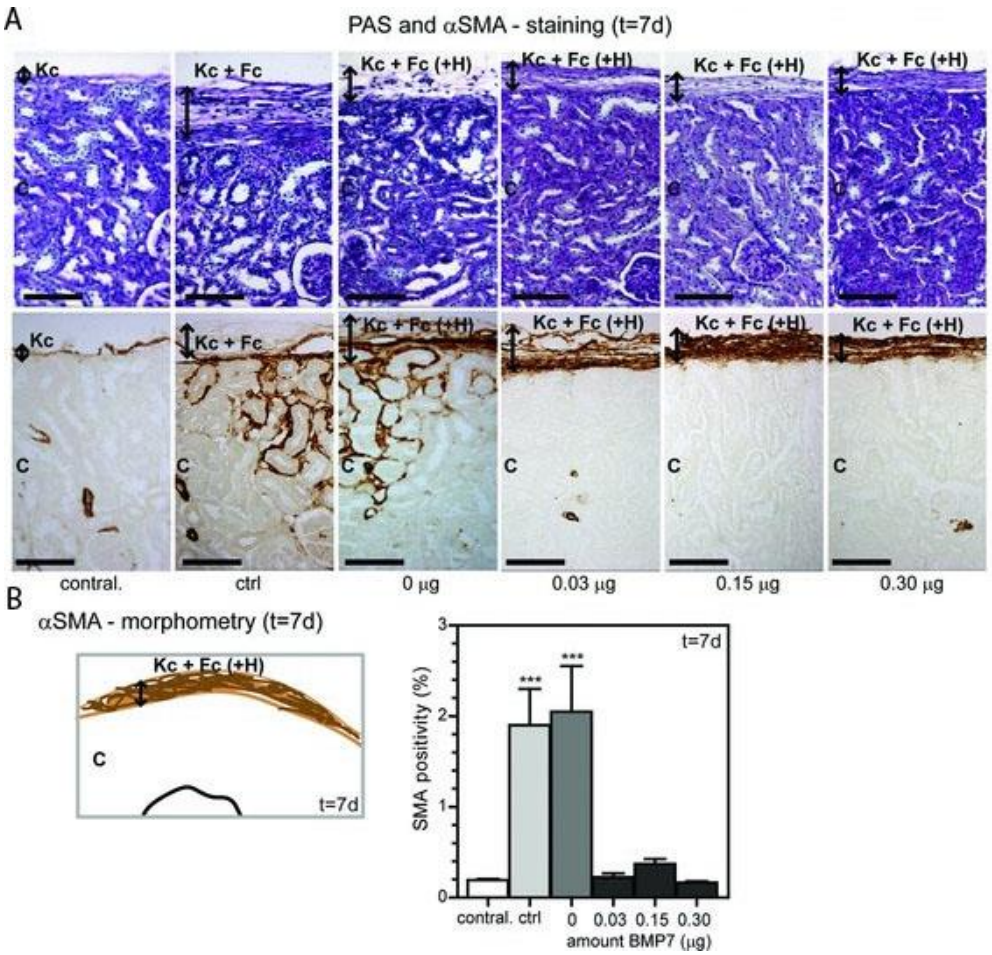

Figure 5 A Modular Injectable Supramolecular Delivery System: In-vivo growth factor delivery in the kidney. (A) After 7 days, the effect of BMP7 delivery under the renal capsule from the hydrogels was studied in the kidney cortex (C). The hydrogel was eroded and a thick capsule was formed which is proposed to be composed of the kidney capsule (Kc), the fibrous capsule (Fc) and possible remnants of the hydrogel (H), indicated with (Kc +Fc (+H)). The kidney morphology was evaluated with a periodic acid Schiff base (PAS) staining. As references, the contralateral kidney

(contral.) and the kidney with only saline (ctrl) are shown. The presence of myofibroblasts in the renal cortex was evaluated with an α-smooth muscle actin (α-SMA) staining. All scale bars represent 100 µm. (B) Schematic representation of the cortex at the site of implantation after 7 days. Morphometry was used to quantify the presence of myofibroblasts in the renal cortex using the α-SMA staining. Modified from Dankers et al. and reproduced with permission of the publisher.[154]

5.2. Smart biomedical devices using shape-memory polymers (SMPs)

Shape-memory polymers (SMPs) are able to memorize a macroscopic shape, exist in a temporary shape under specific conditions of temperature, stress and environmental stimuli, and then relax to the original permanent, stress-free condition;[155, 156] and SMPs have been used as functional textiles,[157] active aircraft equipment,[158] interactive electronic apparatuses,[159] and adaptive biomedical devices.[160, 161]

SMPs are typically classified by the type of stimulus which elicits a physicochemical response (e.g. temperature,[162] solvation,[163] pH,[164] electrical fields,[165, 166] magnetic fields[167] or light); and as well as stimuli descriptors, SMPs can be further classified by their material properties, i.e. polymer crystallinity index, crosslinking profile, mechanism responsible for the shape-memory effect,[168] and to predict responsiveness the architecture of the polymer needs to be considered with respect to the presence of chemical/physical crosslinks, yielding thermoset or thermoplastic polymers, respectively.[169] Physically crosslinked polymers show reversible interaction, and can be either amorphous or semicrystalline (i.e. the transition temperature is either a glass transition temperature (*Tg*), or a melting point (*Tm*)[169-171]), however, the covalent bonds present in thermoplastic polymers connecting switching components can be difficult to reverse. Moreover, the shape-memory effect of polymer systems are attributed to the microphase separation of the net-points, which depends on the molecular weight and composition of the crosslinked segments.[170] Based on the nature of the net-point and switching components, thermally activated SMPs can be categorized into four main groups:[172] 1) physically crosslinked net-points with amorphous molecular switches; 2) physically crosslinked net-points with semicrystalline molecular switches; 3) chemically crosslinked net-points with amorphous molecular switches; 4) chemically crosslinked net-points with semicrystalline molecular switches.[171] The first two groups are thermoplastics and the last two are thermosetting SMPs,[173] examples of which are depicted in **Figure 6**.

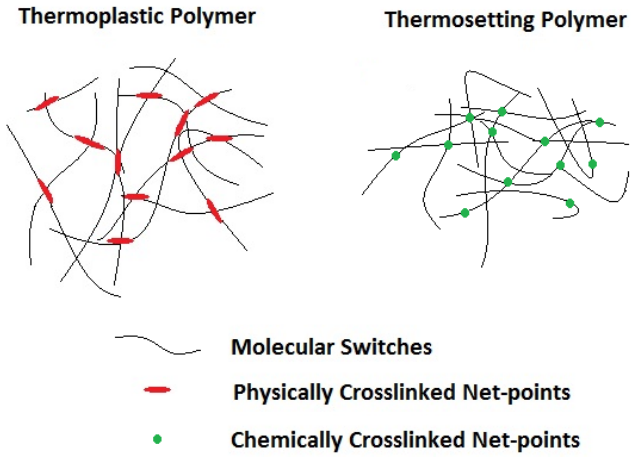

Figure 6. Schematic of thermoplastic and thermosetting polymer networks. Modified from Liu et al. and reproduced with permission of the publisher.[174]

In thermoplastic SMPs, hard segments are responsible for chain-chain interactions via hydrogen bonding or dipole-dipole interactions. Good moldability, high modulus below T_{trans} and high deformability are some advantages of physically crosslinked block copolymers.[171] However, the molecular weight of the polymer chains needs to be high enough for entanglement or phase separation.[171] Certain polyurethanes (PU) show promise as SMPs and have many advantages due to their excellent shape-memory properties and biocompatibility.[169] PUs include urethane bonds (carbamates) generated from the reaction of diisocyanates with glycol chain extenders as hard segments and suitably chosen diisocyanates, commonly polyether chains, as soft segments.[175] Shape-memory properties, transition temperature range and melting point of these smart polymers can be controlled by changing the soft to hard segment ratio.[176]

Chemically crosslinked SMPs have higher stiffness and shape-memory effects, high transition temperature and environmental durability in comparison with thermoplastics, [174] and are prepared either by adding a multi-functional crosslinker during the polymerization or in the post curing process.[177] The shape-memory phenomena in SMPs appear via changing of the chemical structure, the degree of crosslinking and the fraction of amorphous and crystalline domains.[178] Instead of direct heating, thermo-responsive polymers can be triggered to show shape-memory function by applying electricity, magnetism, light, microwave or moisture.[165, 179] With direct triggering, both programming and recovery stages are possible; however, indirect stimuli work only during the recovery process making use of another stimulus necessary for the temporary fixation. Generally, indirect stimuli include water, electric current and magnetic field; whereas light can considered as either a direct or indirect stimulus.[180] In order to enhance the response of SMPs, multiple stimuli can be applied simultaneously or shape changes can be triggered step-by-step.[165, 181]

To exemplify the use of SMPs for biomedical devices, we highlight light-responsive systems,[182] that typically incorporate photo-cleavable units (e.g. cinnamic acid-based molecules) or photoisoimerizable units (e.g. azobenzenes)[180] as depicted in **Figure 7**. Photo-crosslinkable sites in polymer networks respond to the light of wavelength λ1 and the temporary shape (Figure 4-c) is stable until the polymer is exposed to an appropriate stimulus light,[183] yielding light-actuated biodegradable SMPs that can be applied as smart sutures for wound closure.[184]

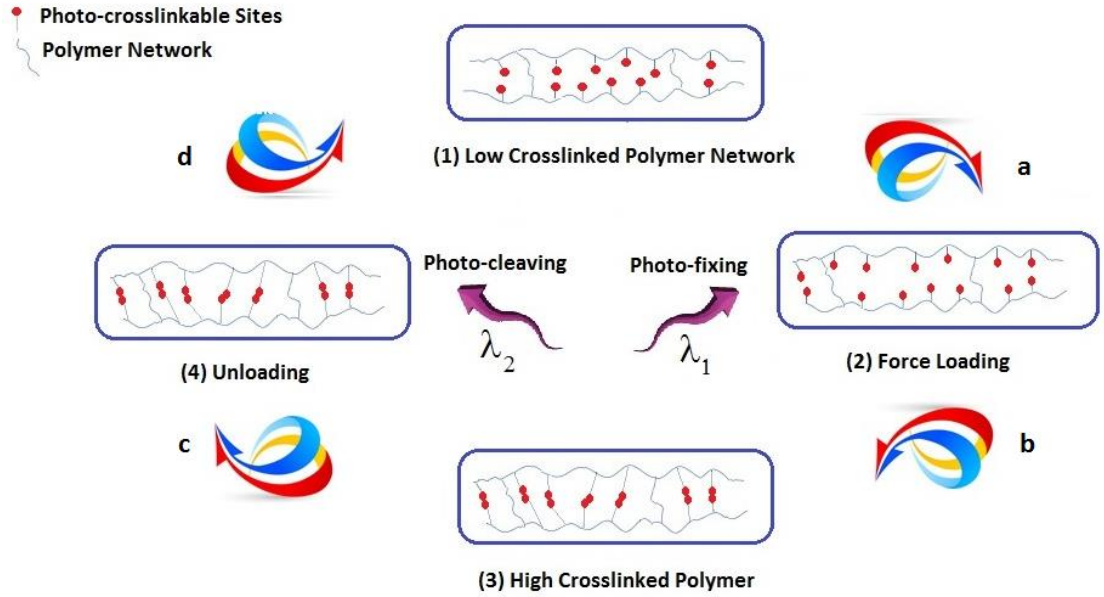

Figure 7. **Schematic of molecular mechanism of photo activated SMP** (a) Deforming polymer by applying stress, (b) photo-fixing with light of wavelength *λ*1, (c) Unloading external stress, (d) photo-cleaving by exposing to light of wavelength *λ*2.

Interest in this field has increased considerably, after the commercial success of SMP products as drug delivery systems and medical devices for minimally invasive surgery has been growing. The main application of these smart materials are listed in **Table 1**. Clearly, changing the chemistry of SMPs in order to modify their biocompatibility, strength, shape recovery and biodegradability are particularly important areas for future research.

Table 1. Applications of shape-memory polymers in regenerative medicine.

Application	Material	Reference
Drug delivery	PCG PCL PLGA PLGA	[185] [155] [156] [157]
Clot removal/ Aneurysm	PU	], 186, 160[158
Suture	Oligo(caprolactone)- diol + oligo(dioxaxonoligo(dioxaxone) + diisocyanates Styrene-butadiene + PCL	][187][168
Stents	PU MM5520 PEG + PCL Crosslinked chitosan+ ethylene glycol diglycidyl ether Block co-polymers PCTBV	], 188[170][189][190
Tissue engineering scaffolds	PGD PCLDMA	], 173[172

6. Future Perspectives

The development of materials for drug delivery and tissue engineering is an exciting multidisciplinary field. The focus of research is beginning to shift from bioinert materials (e.g. gold/mercury amalgam in teeth, titanium or polymethylmethacrylates in bone) towards biomimetic biomaterials, naturally focusing on composite materials produced from hierarchically structured supramolecular assemblies. Interesting examples of drug delivery systems and tissue scaffolds have been produced from these materials, and show promise not only in vitro, but in vivo. New advances in both the understanding of self-organization and the control of matter at the

nanoscale, are bridging the gap between materials and life sciences. In particular, stimuli-responsive and chemically dynamic materials[191] show great promise for the advancement of this field of study and for applications in regenerative medicine.

In addition, while the use of 3D printing technologies for biomedical engineering is still in its nascent stages,[192] we believe that such technologies have great potential for manufacturing the next generation of biomaterials with topographical information on length scales above those currently manageable with supramolecular chemistry alone. This is particularly important because cells respond to topographical cues (i.e. information stored in the topography of the surface that they are interfacing with, particularly as regards cell adhesion, orientation, and migration.[193] Moreover, 3-dimensional (3D) cell culture offers a more realistic micro- and local-environment than 2-dimensional cell culture paradigms.[194] An important factor in the production of working tissue engineering scaffolds is the possibility of a reproducible and controlled method of micro- and sub-micro structuring. A versatile class of the scaffold production techniques which enable the fabrication of tailor made structures directly from computer data via Computer Aided Design / Computer Aided Manufacturing (CAD/CAM), are laser-based Additive Manufacturing (AM) fabrication techniques[195-197]. A number of them have been implemented and commercialized, such as Laser-assisted Bioprinting (LaB), selective laser sintering (SLS), stereolithography (SL) and Direct Laser Writing (DLW) by Multiphoton Polymerization (MPP).

Laser-assisted Bioprinting (LaB)[198, 199, 200] is based on laser-induced forward transfer (LIFT), i.e. the selective forward ablation and deposition of materials using lasers. LaB offers the possibility of printing patterns with a high spatial resolution from a wide range of materials in the solid or liquid state. Here, a laser pulse is focused on a thin film of the material to be transferred (the bio-ink, which can be biomaterials, cells, biomolecules) through a transparent support (**Figure 8A**). A small fraction of the film is transferred to a receptor substrate that is placed parallel to the film-support system, a few microns away.[201] The volume of the material transferred will depend on the optical properties of the bio-ink in respect to the laser properties (wavelength, pulse duration). If the biomaterial is transparent to the laser wavelength, then an intermediate, heat absorbing layer might be needed; usually a thin film of metal such as Au, Cr, Ti, Ag, or a photo-decomposing volatile polymer (e.g. triazene).[200] An almost infinite number of materials can be transferred using LaB, including hydrogels,[202] biomolecules,[203] and living cells,[199, 204] with minimal effect in the materials properties. Importantly, it was demonstrated that it could be used to make 3D structures, such as capillary patterns for vascular engineering,[205] a cardiac patch which included

MSCs and endothelial cells,[206] and skin;[207] and moreover, it is the only laser-based AM technique able to directly print biomaterials and cells simultaneously.

Differently, SLS employs a high power laser to sinter layers of material in powder form in order to build 3D structures as depicted in **Figure 8B**. SLS has been extensively used to create replication masters for biomedical implants;[208] hard scaffolds for bone and cartilage tissue engineering,[209] often employing mixtures of poly-ε-caprolactone (**Figure 8C and 8D**) with calcium phosphates to more accurately mimic bone;[210] and for soft tissue engineering it has been used to prepare poly-ε-caprolactone-based cardiac[211] tissue scaffolds.

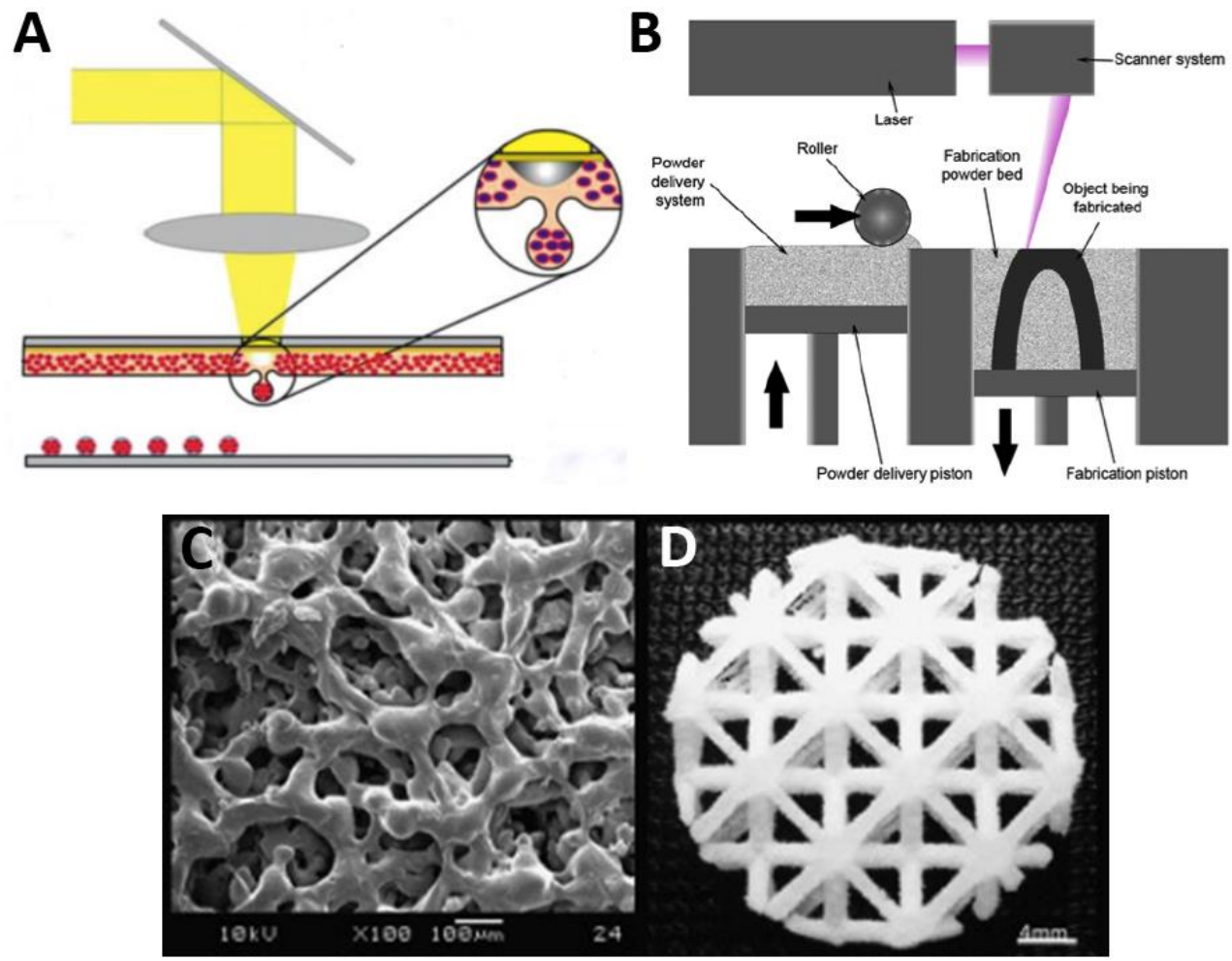

Figure 8 Schematic of laser assisted bioprinting (LaB) and selective laser melting systems (A) LaB offers the possibility of printing patterns with a high spatial resolution from a wide range of materials in the solid or liquid state. Here, a laser pulse is focused on a thin film of the bio-ink. A small fraction of the film is transferred to a receptor substrate that is placed parallel to the film-support system, a few microns away. (B) **Selective laser melting systems** employ a high power laser to sinter layers of material in powder form, in order to build 3D structures in a sequential manner. (C,D) High and low magnification SEM images of a poly-ε-caprolactone scaffolds made using SLS for cardiac regeneration. Modified from Yeong et al. and reproduced with permission of the publisher.[212]

SLA employs a UV laser to polymerize UV-curable liquid resin precursors[213] layer by layer[214] with either commercially available or custom-made apparatuses,[214, 215] using UV-curable derivatives of

poly(propylene fumarate),[216] polylactide,[217] poly-ε-caprolactone,[218] etc.[219] and it has been used to prepare soft tissue scaffolds for aortic valves,[220] microvascular networks,[221] and trachea.[222] Current limitations of SLA are the resolution and availability of materials which are photostructurable, biocompatible and biodegradable, which are areas for future research.

Interestingly, Direct Laser Writing (DLW) by Multi-Photon Polymerization (MPP), as depicted in **Figure 9A**, allows the construction of structures with sub-100 nm resolution,[197, 223] albeit at the expense of writing speed which has limited the size of the constructs prepared by DLW to small scale cell studies, as opposed to whole organ printing.[195] A variety of materials can be employed to make hard, soft and even composite materials,[224] including polymers and proteins as pioneered by Campagnola and *et al.* with bovine serum albumin (BSA), fibrogen, fibronectin, and collagen.[225] Subsequently structures have been printed using biopolymers such as gelatin[226] and hyaluronic acid,[227] or synthetic polymers polycaprolactone,[228] or polylactide (**Figure 9B**).[229, 230] Moreover, it is possible to use DLW/MLP to print within another structure, as exemplified by printing proteins inside a hydrogel for neural cell guidance,[231] and excitingly to encapsulate a live *C-elegans* worm in a protein-based hydrogel.[232]

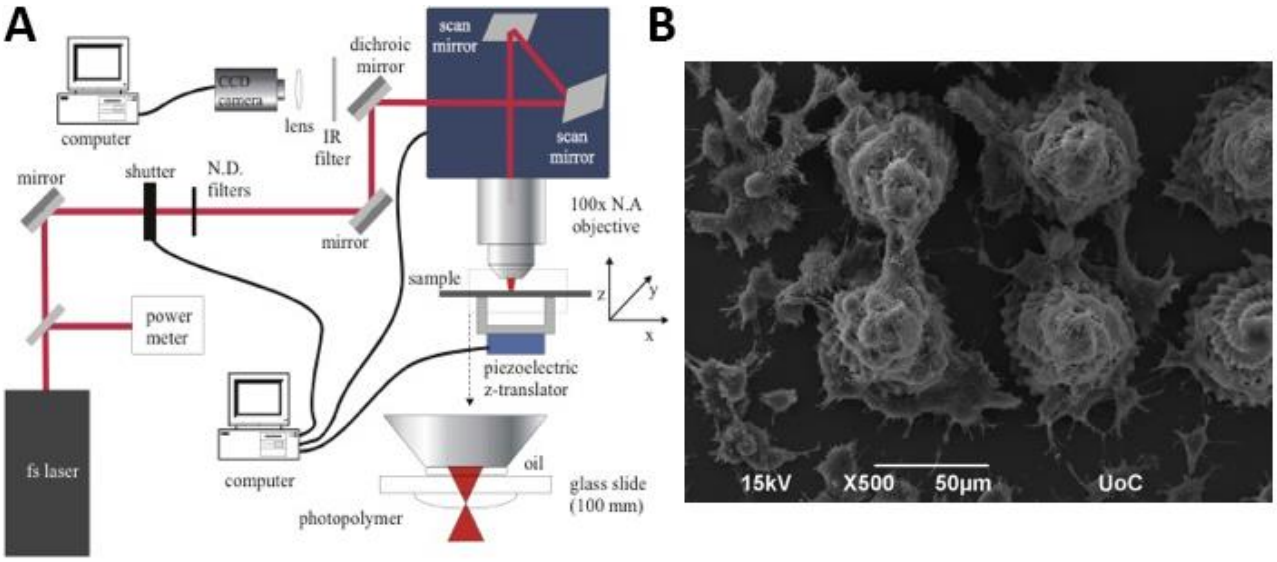

Figure 9 Direct Laser Writing by Multiphoton Polymerization (A) Scaffolds or device morphology is delineated via computer aided design software. These designs are transferred into command signals to control a beam of a sub-picosecond length pulse laser is focused inside the volume of a transparent photosensitive material, causing it to absorb two or more photons and polymerize locally. (B) PC-12 pheochromocytoma cells growing on polylactide structures fabricated via direct laser writing by multiphoton polymerization. Modified from Melissinaki et al. and reproduced with permission of the publisher.[229]

In order to achieve sub-100 nm spatial resolution, different patterning approaches have been employed in recent years.[233] Electron beam (e-beam) lithography[234] has been for example used for patterning proteins[235] and DNA[236] with high fidelity. E-beam uses high-energy (typically several tens of keV) electrons to ablate an electron-sensitive substrate (commonly known as a

resist), depicted in **Figure 10A**, with a resolution down to the sub-5 nm regime, **Figure 10B**. This versatile technique has been used to generate nanoscale patterns of a functional supramolecular "host" templates composed of diaminotriazine-functionalized polystyrene upon which thymine-functionalized CdSe-ZnS QDs assemble via complementary hydrogen-bonding interactions (**Figure 11**),[237] and other studies have focused on cell-substrate interactions that determine contact guidance,[238] adhesion[239] and differential cell behaviour[240] in response to the substrates.

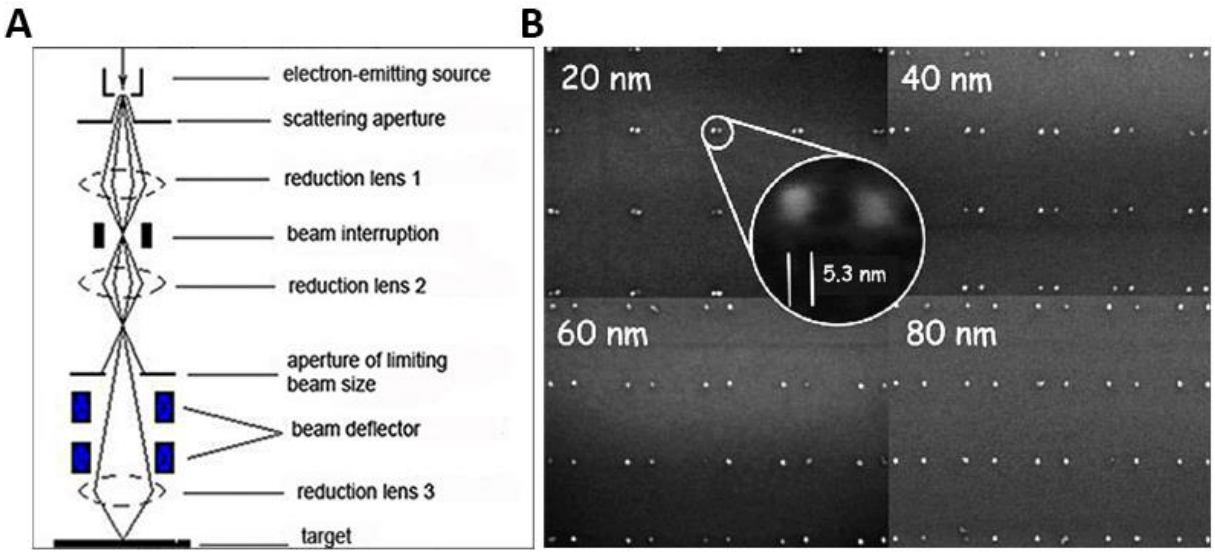

Figure 10. An overview of the Electron-beam system and system resolutions. (A) Electron beam column optics showing the main column and deflection components. (B) Pairs of HSQ nanodots at different inter-pair spacings (indicated in each panel). The dots are ~ 5 nm. Modified from Melissinaki et al. and reproduced with permission of the publisher.[241]

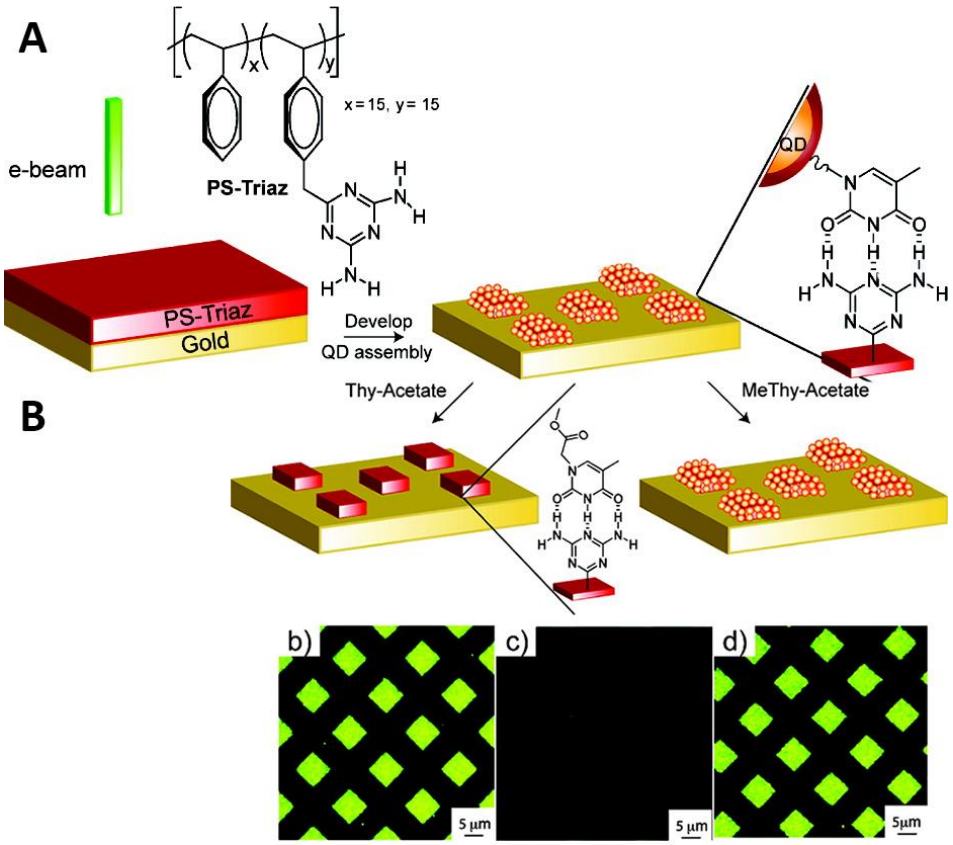

Figure 11 Electron-beam assisted fabrication of functional supramolecular arrays of thymine functionalised quantum dots. (A) Creation of nanopatterned PS-Triaz templates using Electron-beam lithography and functionalization of these features using the complementary Thy-QDs**. (B)** A "lock and key" disassembly of Thy-QDs using thymine acetate methyl ester with an N-methyl thymine acetate methyl ester control. (b) Fluorescent image of patterns after the assembly of Thy-QDs. (c) Fluorescent image after incubation in thymine acetate solution, showing the complete erasure of fluorescence. (d) Fluorescent image of after incubation in N-methylated thymine acetate solution showing no visible change in fluorescence. Modified from Subramani et al. and reproduced with permission of the publisher.[237]

In addition to the aforoemtioned apporaches , nano- and micro-imprint lithography have been key technologies for investigating the role of topographical information on cell-substrate interactions. E-beam generated structures can be reliably transferred into biological and polymeric materials through imprint lithography which can be employed as a high-throughput technique for patterning of proteins[242], peptides[243], and synthetic polymers[240] with high fidelity.

Imprinted arrays of topographical and biochemical patterns have been utilized extensively in vitro to modulate the phenotype[244] of primary cells and to enhance differentiation of stem cells towards osteogenic,[245] neurogenic,[246] adipogenic[247] and myogenic[248] lineages. Indeed, arrays of RGD-functionalized sub-10 nm metal dots were employed to study the role of the geometric organization of extracellular matrix (ECM) binding ligands on the adhesion and spreading of fibroblasts, identifying a minimum cell spreading adhesive unit that involves the clustering of at least 4 integrins within ~60 nm.[249] Interestingly, stem cells have been shown to maintain their stem cell markers on patterned substrates for up to eight weeks in vitro culture without

phonetypic drift,[240] and even when cells were cultured in osteodifferentiation media only modest levels of osteogenic markers were noted.

Although both lithographic and additive manufacturing techniques have generated an abundance of interesting studies performed *in vitro,* these technologies have yet to be effectively translated into clinical advances, and the concept of controlled morphological and topographical modification in tissue engineering has not produced compelling data to date in preclinical studies. However, we believe 3D printing technologies represent the future of manufacturing patient-specific biomaterials for drug delivery and tissue engineering;[192, 250] and moreover that components produced by the field of synthetic biology (e.g. engineered proteins, DNA, polymer-biomolecule hybrids) will play an increasingly large role as the building blocks of drug delivery devices and tissue scaffolds in the (near) future. [110, 251]

Acknowledgements

Maria Farsari acknowledges financial support from the THALIS Program 3DSET (MIS380278) of the Hellenic Ministry of Education, and the FP7 Marie Curie ITN Program **AngioMatTrain** (Grant Agreement no. 317304). M.J. Biggs is a Science Foundation Ireland, Starting Investigator SIRG COFUND fellow (grant agreement no. 11/SIRG/B2135), and a funded investigator through the Science Foundation Ireland Centre for Research in Medical Devices (CÚRAM) (Grant agreement no. 13/RC/2073)

References

[1] S. T. Nguyen, D. L. Gin, J. T. Hupp, X. Zhang, Proceedings of the National Academy of Sciences of the United States of America 2001, 98, 11849.
[2] E. Busseron, Y. Ruff, E. Moulin, N. Giuseppone, Nanoscale 2013, 5, 7098.
[3] N. C. Seeman, J Theor Biol 1982, 99, 237.
[4] Y. W. Zhang, N. C. Seeman, J Am Chem Soc 1992, 114, 2656.
[5] J. Chen, N. C. Seeman, Abstr Pap Am Chem S 1991, 202, 162.
[6] Y. W. Zhang, N. C. Seeman, J Am Chem Soc 1994, 116, 1661; Y. W. Zhang, N. C. Seeman, Biophys J 1994, 66, A24.
[7] E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, Nature 1998, 394, 539.
[8] H. Yan, T. H. LaBean, L. P. Feng, J. H. Reif, Proceedings of the National Academy of Sciences of the United States of America 2003, 100, 8103.

[9] R. D. Barish, R. Schulman, P. W. K. Rothemund, E. Winfree, Proceedings of the National Academy of Sciences of the United States of America 2009, 106, 6054.
[10] R. P. Goodman, R. M. Berry, A. J. Turberfield, Chem Commun 2004, 1372; Y. He, T. Ye, M. Su, C. Zhang, A. E. Ribbe, W. Jiang, C. D. Mao, Nature 2008, 452, 198; W. M. Shih, J. D. Quispe, G. F. Joyce, Nature 2004, 427, 618.
[11] P. J. Paukstelis, J. Nowakowski, J. J. Birktoft, N. C. Seeman, Chemistry & Biology 2004, 11, 1119.
[12] J. Zheng, J. J. Birktoft, Y. Chen, T. Wang, R. Sha, P. E. Constantinou, S. L. Ginell, C. Mao, N. C. Seeman, Nature 2009, 461, 74.
[13] N. C. Seeman, Annual review of biochemistry 2010, 79, 65.
[14] P. W. K. Rothemund, Nature 2006, 440, 297.
[15] While the term "DNA origami" was first coined by Rothemund to describe the folding of a single ssDNA scaffold strand, it has come to refer to nearly all forms of DNA nanostructures, including the smaller tiles and their derivatives.
[16] M. Rahman, D. Neff, M. L. Norton, Chem Commun 2014, 50, 3413.
[17] W. Liu, H. Zhong, R. Wang, N. C. Seeman, Angewandte Chemie International Edition 2011, 50, 264.
[18] Z. Zhao, Y. Liu, H. Yan, Nano Letters 2011, 11, 2997.
[19] P. K. Lo, K. L. Metera, H. F. Sleiman, Current Opinion in Chemical Biology 2010, 14, 597.
[20] E. S. Andersen, M. Dong, M. M. Nielsen, K. Jahn, R. Subramani, W. Mamdouh, M. M. Golas, B. Sander, H. Stark, C. L. P. Oliveira, J. S. Pedersen, V. Birkedal, F. Besenbacher, K. V. Gothelf, J. Kjems, Nature 2009, 459, 73.
[21] S. M. Douglas, H. Dietz, T. Liedl, B. Hogberg, F. Graf, W. M. Shih, Nature 2009, 459, 414.
[22] H. Dietz, S. M. Douglas, W. M. Shih, Science 2009, 325, 725.
[23] D. R. Han, S. Pal, J. Nangreave, Z. T. Deng, Y. Liu, H. Yan, Science 2011, 332, 342.
[24] K. Sanderson, Nature 2010, 464, 158.
[25] F. Zhang, J. Nangreave, Y. Liu, H. Yan, J Am Chem Soc 2014, 136, 11198.
[26] N. C. Seeman, J Biomol Struct Dyn 1990, 8, 573.
[27] H. Y. Li, S. H. Park, J. H. Reif, T. H. LaBean, H. Yan, J Am Chem Soc 2004, 126, 418.
[28] J. W. Zheng, P. E. Constantinou, C. Micheel, A. P. Alivisatos, R. A. Kiehl, N. C. Seeman, Nano Letters 2006, 6, 1502; Y. Y. Pinto, J. D. Le, N. C. Seeman, K. Musier-Forsyth, T. A. Taton, R. A. Kiehl, Nano Letters 2005, 5, 2399; J. D. Le, Y. Pinto, N. C. Seeman, K. Musier-Forsyth, T. A. Taton, R. A. Kiehl, Nano Letters 2004, 4, 2343; S. J. Xiao, F. R. Liu, A. E. Rosen, J. F. Hainfeld, N. C. Seeman, K. Musier-Forsyth, R. A. Kiehl, J Nanopart Res 2002, 4, 313.
[29] J. Sharma, Y. Ke, C. Lin, R. Chhabra, Q. Wang, J. Nangreave, Y. Liu, H. Yan, Angewandte Chemie International Edition 2008, 47, 5157.
[30] B. Högberg, H. Olin, Journal of Physics: Conference Series 2007, 61, 458; S. Pal, Z. Deng, B. Ding, H. Yan, Y. Liu, Angewandte Chemie International Edition 2010, 49, 2700.
[31] H. Bui, C. Onodera, C. Kidwell, Y. Tan, E. Graugnard, W. Kuang, J. Lee, W. B. Knowlton, B. Yurke, W. L. Hughes, Nano Letters 2010, 10, 3367.
[32] H. T. Maune, S.-p. Han, R. D. Barish, M. Bockrath, I. I. A. Goddard, W. K. RothemundPaul, E. Winfree, Nat Nano 2010, 5, 61.
[33] S. Rinker, Y. Ke, Y. Liu, R. Chhabra, H. Yan, Nat Nano 2008, 3, 418.
[34] Y. He, Y. Tian, A. E. Ribbe, C. Mao, J Am Chem Soc 2006, 128, 12664; F. R. Liu, R. J. Sha, N. C. Seeman, J Am Chem Soc 1999, 121, 917; H. Yan, S. H. Park, G. Finkelstein, J. H. Reif, T. H. LaBean, Science 2003, 301, 1882.
[35] A. Y. Koyfman, G. B. Braun, N. O. Reich, J Am Chem Soc 2009, 131, 14237.
[36] S. Bi, Y. Dong, X. Jia, M. Chen, H. Zhong, B. Ji, Nanoscale 2015, 7, 7361; Y. H. Roh, J. B. Lee, K. E. Shopsowitz, E. C. Dreaden, S. W. Morton, Z. Poon, J. Hong, I. Yamin, D. K. Bonner, P. T. Hammond, ACS nano 2014, 8, 9767; W. Sun, Z. Gu, Biomaterials Science 2015, 3, 1018.

[37] N. Stephanopoulos, R. Freeman, H. A. North, S. Sur, S. J. Jeong, F. Tantakitti, J. A. Kessler, S. I. Stupp, Nano Letters 2015, 15, 603.
[38] J. Li, C. H. Fan, H. Pei, J. Y. Shi, Q. Huang, Advanced materials 2013, 25, 4386.
[39] Q. Mei, X. Wei, F. Su, Y. Liu, C. Youngbull, R. Johnson, S. Lindsay, H. Yan, D. Meldrum, Nano Letters 2011, 11, 1477; X. Shen, Q. Jiang, J. Wang, L. Dai, G. Zou, Z.-G. Wang, W.-Q. Chen, W. Jiang, B. Ding, Chem Commun 2012, 48, 11301.
[40] S. Kocabey, H. Meinl, I. S. MacPherson, V. Cassinelli, A. Manetto, S. Rothenfusser, T. Liedl, F. S. Lichtenegger, Nanomaterials-Basel 2015, 5, 47.
[41] G. D. Hamblin, K. M. M. Carneiro, J. F. Fakhoury, K. E. Bujold, H. F. Sleiman, J Am Chem Soc 2012, 134, 5426.
[42] A. H. Okholm, J. S. Nielsen, M. Vinther, R. S. Sorensen, D. Schaffert, J. Kjems, Methods 2014, 67, 193.
[43] A. S. Walsh, H. F. Yin, C. M. Erben, M. J. A. Wood, A. J. Turberfield, ACS nano 2011, 5, 5427.
[44] L. Liang, J. Li, Q. Li, Q. Huang, J. Y. Shi, H. Yan, C. H. Fan, Angew Chem Int Edit 2014, 53, 7745.
[45] J. Hahn, S. F. J. Wickham, W. M. Shih, S. D. Perrault, ACS nano 2014, 8, 8765.
[46] S. Sellner, S. Kocabey, K. Nekolla, F. Krombach, T. Liedl, M. Rehberg, Biomaterials 2015, 53, 453.
[47] J. Li, H. Pei, B. Zhu, L. Liang, M. Wei, Y. He, N. Chen, D. Li, Q. Huang, C. H. Fan, ACS nano 2011, 5, 8783.
[48] J. Li, C. Zheng, S. Cansiz, C. Wu, J. Xu, C. Cui, Y. Liu, W. Hou, Y. Wang, L. Zhang, I. T. Teng, H. H. Yang, W. Tan, J Am Chem Soc 2015, 137, 1412.
[49] Q. Zhang, Q. Jiang, N. Li, L. R. Dai, Q. Liu, L. L. Song, J. Y. Wang, Y. Q. Li, J. Tian, B. Q. Ding, Y. Du, ACS nano 2014, 8, 6633.
[50] A. Gopinath, P. W. K. Rothemund, ACS nano 2014, 8, 12030; R. S. Wang, M. Palma, E. Penzo, S. J. Wind, Nano Res 2013, 6, 409.
[51] Y. Suzuki, M. Endo, H. Sugiyama, ACS nano 2015.
[52] S. M. Douglas, I. Bachelet, G. M. Church, Science 2012, 335, 831.
[53] Y.-X. Zhao, A. Shaw, X. Zeng, E. Benson, A. M. Nystrom, B. Hogberg, ACS nano 2012, 6, 8684.
[54] S. Modi, C. Nizak, S. Surana, S. Halder, Y. Krishnan, Nature Nanotechnology 2013, 8, 459.
[55] X. Liu, Y. Xu, T. Yu, C. Clifford, Y. Liu, H. Yan, Y. Chang, Nano Letters 2012, 12, 4254.
[56] H. Lee, A. K. R. Lytton-Jean, Y. Chen, K. T. Love, A. I. Park, E. D. Karagiannis, A. Sehgal, W. Querbes, C. S. Zurenko, M. Jayaraman, C. G. Peng, K. Charisse, A. Borodovsky, M. Manoharan, J. S. Donahoe, J. Truelove, M. Nahrendorf, R. Langer, D. G. Anderson, Nature Nanotechnology 2012, 7, 389.
[57] H. Pei, L. Liang, G. B. Yao, J. Li, Q. Huang, C. H. Fan, Angew Chem Int Edit 2012, 51, 9020.
[58] Y. Amir, E. Ben-Ishay, D. Levner, S. Ittah, A. Abu-Horowitz, I. Bachelet, Nature Nanotechnology 2014, 9, 353.
[59] S. G. Zhang, Nat Biotechnol 2003, 21, 1171.
[60] P. Kumar, V. Pillay, G. Modi, Y. E. Choonara, L. C. du Toit, D. Naidoo, Recent patents on drug delivery & formulation 2011, 5, 24.
[61] R. V. Ulijn, D. N. Woolfson, Chem Soc Rev 2010, 39, 3349.
[62] X. B. Zhao, F. Pan, H. Xu, M. Yaseen, H. H. Shan, C. A. E. Hauser, S. G. Zhang, J. R. Lu, Chem Soc Rev 2010, 39, 3480; P. A. Korevaar, C. J. Newcomb, E. W. Meijer, S. I. Stupp, J Am Chem Soc 2014, 136, 8540.
[63] H. G. Cui, M. J. Webber, S. I. Stupp, Biopolymers 2010, 94, 1; J. H. Collier, J. S. Rudra, J. Z. Gasiorowski, J. P. Jung, Chem Soc Rev 2010, 39, 3413.
[64] E. Arslan, I. C. Garip, G. Gulseren, A. B. Tekinay, M. O. Guler, Adv Healthc Mater 2014, 3, 1357.

[65] S. Maude, E. Ingham, A. Aggeli, Nanomedicine-Uk 2013, 8, 823.
[66] M. Zelzer, R. V. Ulijn, Chem Soc Rev 2010, 39, 3351; D. W. P. M. Lowik, E. H. P. Leunissen, M. van den Heuvel, M. B. Hansen, J. C. M. van Hest, Chem Soc Rev 2010, 39, 3394.
[67] J. J. Panda, V. S. Chauhan, Polym Chem-Uk 2014, 5, 4418; S. G. Zhang, Accounts Chem Res 2012, 45, 2142.
[68] Z. L. Luo, S. G. Zhang, Chem Soc Rev 2012, 41, 4736.
[69] J. B. Matson, R. H. Zha, S. I. Stupp, Curr Opin Solid St M 2011, 15, 225.
[70] E. De Santis, N. Faruqui, J. E. Noble, M. G. Ryadnov, Nanoscale 2014, 6, 11425.
[71] N. Faruqui, A. Bella, J. Ravi, S. Ray, B. Lamarre, M. G. Ryadnov, J Am Chem Soc 2014, 136, 7889.
[72] G. A. Silva, C. Czeisler, K. L. Niece, E. Beniash, D. A. Harrington, J. A. Kessler, S. I. Stupp, Science 2004, 303, 1352.
[73] V. M. Tysseling-Mattiace, V. Sahni, K. L. Niece, D. Birch, C. Czeisler, M. G. Fehlings, S. I. Stupp, J. A. Kessler, J Neurosci 2008, 28, 3814.
[74] B. Mammadov, R. Mammadov, M. O. Guler, A. B. Tekinay, Acta Biomater 2012, 8, 2077.
[75] S. Koutsopoulos, S. G. Zhang, Acta Biomater 2013, 9, 5162.
[76] B. He, X. Yuan, D. M. Jiang, Rsc Adv 2014, 4, 23610.
[77] A. J. Lomas, C. N. Ryan, A. Sorushanova, N. Shologu, A. I. Sideri, V. Tsioli, G. C. Fthenakis, A. Tzora, I. Skoufos, L. R. Quinlan, G. O'Laighin, A. M. Mullen, J. L. Kelly, S. Kearns, M. Biggs, A. Pandit, D. I. Zeugolis, Advanced drug delivery reviews 2014.
[78] N. L. Angeloni, C. W. Bond, Y. Tang, D. A. Harrington, S. M. Zhang, S. I. Stupp, K. E. McKenna, C. A. Podlasek, Biomaterials 2011, 32, 1091.
[79] C. C. Huang, S. Ravindran, Z. Y. Yin, A. George, Biomaterials 2014, 35, 5316.
[80] B. He, X. Yuan, A. G. Zhou, H. Zhang, D. M. Jiang, Expert Rev Mol Med 2014, 16.
[81] R. N. Shah, N. A. Shah, M. M. D. Lim, C. Hsieh, G. Nuber, S. I. Stupp, Proceedings of the National Academy of Sciences of the United States of America 2010, 107, 3293.
[82] M. J. Webber, J. Tongers, C. J. Newcomb, K. T. Marquardt, J. Bauersachs, D. W. Losordo, S. I. Stupp, Proceedings of the National Academy of Sciences of the United States of America 2012, 109, 9220.
[83] R. H. Zha, S. Sur, J. Boekhoven, H. Y. Shi, M. Zhang, S. I. Stupp, Acta Biomater 2015, 12, 1.
[84] V. A. Kumar, N. L. Taylor, S. Shi, B. K. Wang, A. A. Jalan, M. K. Kang, N. C. Wickremasinghe, D. Hartgerink, ACS nano 2015, 9, 860.
[85] M. I. Bury, N. J. Fuller, J. W. Meisner, M. D. Hofer, M. J. Webber, L. W. Chow, S. Prasad, H. Thaker, X. Yue, V. S. Menon, E. C. Diaz, S. I. Stupp, E. Y. Cheng, A. K. Sharma, Biomaterials 2014, 35, 9311.
[86] J. B. Matson, S. I. Stupp, Chem Commun 2012, 48, 26.
[87] M. J. Webber, X. Q. Han, S. N. P. Murthy, K. Rajangam, S. I. Stupp, J. W. Lomasney, J Tissue Eng Regen M 2010, 4, 600.
[88] Y. Kuang, J. F. Shi, J. Li, D. Yuan, K. A. Alberti, Q. B. Xu, B. Xu, Angew Chem Int Edit 2014, 53, 8104.
[89] C. J. Newcomb, S. Sur, J. H. Ortony, O. S. Lee, J. B. Matson, J. Boekhoven, J. M. Yu, G. C. Schatz, S. I. Stupp, Nat Commun 2014, 5.
[90] L. W. Chow, A. Armgarth, J. P. St-Pierre, S. Bertazzo, C. Gentilini, C. Aurisicchio, S. D. McCullen, J. A. M. Steele, M. M. Stevens, Adv Healthc Mater 2014, 3, 1381.
[91] R. M. Capito, H. S. Azevedo, Y. S. Velichko, A. Mata, S. I. Stupp, Science 2008, 319, 1812.
[92] R. H. Zha, S. Sur, S. I. Stupp, Adv Healthc Mater 2013, 2, 631.
[93] A. C. Mendes, K. H. Smith, E. Tejeda-Montes, E. Engel, R. L. Reis, H. S. Azevedo, A. Mata, Adv Funct Mater 2013, 23, 430.
[94] D. S. Ferreira, Y.-A. Lin, H. G. Cui, J. A. Hubbell, R. L. Reis, H. S. Azevedo, Adv Healthc Mater 2015, 4, 602.

[95] S. K. Nitta, K. Numata, Int J Mol Sci 2013, 14, 1629; Q. Yan, J. Yuan, Z. Cai, Y. Xin, Y. Kang, Y. Yin, J Am Chem Soc 2010, 132, 9268.
[96] M. Young, D. Willits, M. Uchida, T. Douglas, Annu Rev Phytopathol 2008, 46, 361; Z. Liu, J. Qiao, Z. W. Niu, Q. Wang, Chem Soc Rev 2012, 41, 6178; M. T. Dedeo, D. T. Finley, M. B. Francis, Prog Mol Biol Transl 2011, 103, 353.
[97] N. Ferrer-Miralles, E. Rodriguez-Carmona, J. L. Corchero, E. Garcia-Fruitos, E. Vazquez, A. Villaverde, Critical reviews in biotechnology 2013; E. Vazquez, A. Villaverde, Microbial cell factories 2010, 9, 101.
[98] N. Stephanopoulos, G. J. Tong, S. C. Hsiao, M. B. Francis, ACS nano 2010, 4, 6014.
[99] W. Wu, S. C. Hsiao, Z. M. Carrico, M. B. Francis, Angew Chem Int Edit 2009, 48, 9493.
[100] X. Zhao, Y. Lin, Q. Wang, Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology 2014.
[101] H. N. Vu, J. D. Ramsey, D. W. Pack, Molecular therapy : the journal of the American Society of Gene Therapy 2008, 16, 308; J. D. Ramsey, H. N. Vu, D. W. Pack, Journal of controlled release : official journal of the Controlled Release Society 2010, 144, 39; R. K. Keswani, I. M. Pozdol, D. W. Pack, Molecular pharmaceutics 2013, 10, 1725.
[102] S. M. DePorter, B. R. McNaughton, Bioconjugate Chem 2014, 25, 1620.
[103] N. Stephanopoulos, G. J. Tong, S. C. Hsiao, M. B. Francis, ACS nano 2010, 4, 6014.
[104] Q. S. Liu, J. Jung, M. Somiya, M. Iijima, N. Yoshimoto, T. Niimi, A. D. Maturana, S. H. Shin, S. Y. Jeong, E. K. Choi, S. Kuroda, Int J Nanomed 2015, 10, 4159.
[105] T. Kato, M. Yui, V. Deo, E. Park, Pharm Res. 2015.
[106] P. Anand, A. O'Neil, E. Lin, T. Douglas, M. Holford, Scientific Reports 2015, 5.
[107] L. Kirscher, X. L. Dean-Ben, M. Scadeng, A. Zaremba, Q. Zhang, C. Kober, T. F. Fehm, D. Razansky, V. Ntziachristos, J. Stritzker, A. A. Szalay, Theranostics 2015, 5, 1045.
[108] G. K. Gruenwald, A. Vetter, K. Klutz, M. J. Willhauck, N. Schwenk, R. Senekowitsch-Schmidtke, M. Schwaiger, C. Zach, E. Wagner, B. Goeke, P. S. Holm, M. Ogris, C. Spitzweg, Molecular Therapy-Nucleic Acids 2013, 2; W. H. Chen, Y. H. Cao, M. Liu, Q. H. Zhao, J. Huang, H. L. Zhang, Z. W. Deng, J. W. Dai, D. F. Williams, Z. J. Zhang, Biomaterials 2012, 33, 7895; J. J. Rojas, S. H. Thorne, Theranostics 2012, 2, 363.
[109] J. A. Luckanagul, L. A. Lee, S. You, X. Yang, Q. Wang, Journal of Biomedical Materials Research Part A 2015, 103, 887.
[110] D. Sengupta, S. C. Heilshorn, Tissue Engineering Part B-Reviews 2010, 16, 285.
[111] J. Velema, D. Kaplan, in *Tissue Engineering I: Scaffold Systems for Tissue Engineering*, Vol. 102, 2006, 187; A. MaHam, Z. Tang, H. Wu, J. Wang, Y. Lin, Small 2009, 5, 1706; G. H. Altman, F. Diaz, C. Jakuba, T. Calabro, R. L. Horan, J. S. Chen, H. Lu, J. Richmond, D. L. Kaplan, Biomaterials 2003, 24, 401; E. Sayin, E. T. Baran, V. Hasirci, Regenerative Medicine 2014, 9, 687.
[112] S. F. Badylak, D. O. Freytes, T. W. Gilbert, Acta Biomater 2009, 5, 1; M. T. Wolf, K. A. Daly, E. P. Brennan-Pierce, S. A. Johnson, C. A. Carruthers, A. D'Amore, S. P. Nagarkar, S. S. Velankar, S. F. Badylak, Biomaterials 2012, 33, 7028; T. W. Gilbert, T. L. Sellaro, S. F. Badylak, Biomaterials 2006, 27, 3675; S. Browne, D. I. Zeugolis, A. Pandit, Tissue Engineering Part A 2013, 19, 1491; A. N. Renth, M. S. Detamore, Tissue Engineering Part B-Reviews 2012, 18, 341.
[113] A. M. Testera, A. Girotti, I. Gonzalez de Torre, L. Quintanilla, M. Santos, M. Alonso, J. Carlos Rodriguez-Cabello, Journal of Materials Science-Materials in Medicine 2015, 26; M. Weber, I. Gonzalez de Torre, R. Moreira, J. Frese, C. Oedekoven, M. Alonso, C. J. Rodriguez Cabello, S. Jockenhoevel, P. Mela, Tissue engineering. Part C, Methods 2015, 21, 832; I. Gonzalez de Torre, F. Wolf, M. Santos, L. Rongen, M. Alonso, S. Jockenhoevel, J. C. Rodriguez-Cabello, P. Mela, Acta Biomater 2015, 12, 146; E. Tejeda-Montes, A. Klymov, M. R. Nejadnik, M. Alonso, J. C. Rodriguez-Cabello, X. F. Walboomers, A. Mata, Biomaterials 2014, 35, 8339.
[114] C. M. Vaz, P. van Doeveren, R. L. Reis, A. M. Cunha, Biomacromolecules 2003, 4, 1520.

[115] Y. Zhang, L. Cui, X. Che, H. Zhang, N. Shi, C. Li, Y. Chen, W. Kong, Journal of Controlled Release 2015, 206, 206.
[116] N. Holten-Andersen, J. H. Waite, Journal of Dental Research 2008, 87, 701; Z. Jiang, Y. Yu, L. Du, X. Ding, H. Xu, Y. Sun, Q. Zhang, Colloid Surface B 2012, 90, 227.
[117] M. L. Lovett, X. Wang, T. Yucel, L. York, M. Keirstead, L. Haggerty, D. L. Kaplan, European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V 2015.
[118] S. Murab, J. Samal, A. Shrivastava, A. R. Ray, A. Pandit, S. Ghosh, Biomaterials 2015, 55, 64.
[119] S. S. Lee, B. J. Huang, S. R. Kaltz, S. Sur, C. J. Newcomb, S. R. Stock, R. N. Shah, S. I. Stupp, Biomaterials 2013, 34, 452.
[120] P. B. Malafaya, G. A. Silva, R. L. Reis, Advanced drug delivery reviews 2007, 59, 207.
[121] A. D. Augst, H. J. Kong, D. J. Mooney, Macromolecular bioscience 2006, 6, 623.
[122] J. Boekhoven, R. H. Zha, F. Tantakitti, E. Zhuang, R. Zandi, C. J. Newcomb, S. I. Stupp, Rsc Adv 2015, 5, 8753.
[123] A. Moshaverinia, X. Xu, C. Chen, K. Akiyama, M. L. Snead, S. Shi, Acta Biomater 2013, 9, 9343.
[124] M. Jorfi, E. J. Foster, J Appl Polym Sci 2015, 132.
[125] N. Lin, A. Dufresne, Biomacromolecules 2013, 14, 871.
[126] M. M. Malinen, L. K. Kanninen, A. Corlu, H. M. Isoniemi, Y. R. Lou, M. L. Yliperttula, A. O. Urtti, Biomaterials 2014, 35, 5110.
[127] T. L. Yang, Int J Mol Sci 2011, 12, 1936.
[128] L. Yin, Z. Song, K. H. Kim, N. Zheng, N. P. Gabrielson, J. Cheng, Advanced materials 2013, 25, 3063.
[129] G. Toskas, S. Heinemann, C. Heinemann, C. Cherif, R. D. Hund, V. Roussis, T. Hanke, Carbohydrate polymers 2012, 89, 997.
[130] J. G. Torres-Rendon, T. Femmer, L. De Laporte, T. Tigges, K. Rahimi, F. Gremse, S. Zafarnia, W. Lederle, S. Ifuku, M. Wessling, J. G. Hardy, A. Walther, Advanced materials 2015, 27, 2989.
[131] N. Li, Y. Chen, Y. M. Zhang, Y. Yang, Y. Su, J. T. Chen, Y. Liu, Sci Rep-Uk 2014, 4; X. Han, Z. Li, J. Sun, C. Luo, L. Li, Y. Liu, Y. Du, S. Qiu, X. Ai, C. Wu, H. Lian, Z. He, Journal of controlled release : official journal of the Controlled Release Society 2015, 197, 29.
[132] K. M. Choi, M. Jang, J. H. Kim, H. J. Ahn, Biomaterials 2014, 35, 7121.
[133] K. M. Park, J. A. Yang, H. Jung, J. Yeom, J. S. Park, K. H. Park, A. S. Hoffman, S. K. Hahn, K. Kim, ACS nano 2012, 6, 2960.
[134] J. Yeom, S. J. Kim, H. Jung, H. Namkoong, J. Yang, B. W. Hwang, K. Oh, K. Kim, Y. C. Sung, S. K. Hahn, Adv Healthc Mater 2015, 4, 237.
[135] H. Jung, J. S. Park, J. Yeom, N. Selvapalam, K. M. Park, K. Oh, J. A. Yang, K. H. Park, S. K. Hahn, K. Kim, Biomacromolecules 2014, 15, 707.
[136] W. A. Braunecker, K. Matyjaszewski, Prog Polym Sci 2007, 32, 93.
[137] J. B. Gilroy, T. Gadt, G. R. Whittell, L. Chabanne, J. M. Mitchels, R. M. Richardson, M. A. Winnik, I. Manners, Nature chemistry 2010, 2, 566.
[138] M. Ujihara, T. Imae, Polym Int 2010, 59, 137.
[139] W. H. Lin, X. G. Guan, T. T. Sun, Y. B. Huang, X. B. Jing, Z. G. Xie, Colloid Surface B 2015, 126, 217.
[140] M. R. Saboktakin, R. M. Tabatabaei, International journal of biological macromolecules 2015, 75, 426; R. Dong, Y. Zhou, X. Huang, X. Zhu, Y. Lu, J. Shen, Advanced materials 2015, 27, 498; J. Thiele, Y. Ma, S. M. Bruekers, S. Ma, W. T. Huck, Advanced materials 2014, 26, 125.
[141] L. G. Griffith, G. Naughton, Science 2002, 295, 1009; J. Boekhoven, S. I. Stupp, Advanced materials 2014, 26, 1642.

[142] J. Li, Npg Asia Mater 2010, 2, 112; J. X. Zhang, P. X. Ma, Advanced drug delivery reviews 2013, 65, 1215.
[143] S. Xu, X. Yang, X. Chen, X. Lin, L. Zhang, G. Yang, C. Gao, Z. Gou, Biomedical materials 2011, 6, 035002.
[144] E. Beniash, Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology 2011, 3, 47.
[145] W. Xiao, W. H. Chen, C. Li, J. X. Chen, X. Z. Zhang, R. X. Zhuo, Journal of biomedical materials research. Part A 2012, 100, 149.
[146] M. Licciardi, D. Paolino, C. Celia, G. Giammona, G. Cavallaro, M. Fresta, Biomaterials 2010, 31, 7340.
[147] M. A. Woodruff, D. W. Hutmacher, Prog Polym Sci 2010, 35, 1217.
[148] S. Zhao, J. Lee, W. Xu, Carbohydrate research 2009, 344, 2201.
[149] H. Cui, L. Cui, P. Zhang, Y. Huang, Y. Wei, X. Chen, Macromolecular bioscience 2014, 14, 440.
[150] P. Y. Dankers, M. C. Harmsen, L. A. Brouwer, M. J. van Luyn, E. W. Meijer, Nature materials 2005, 4, 568.
[151] P. Y. Dankers, E. N. van Leeuwen, G. M. van Gemert, A. J. Spiering, M. C. Harmsen, L. A. Brouwer, H. M. Janssen, A. W. Bosman, M. J. van Luyn, E. W. Meijer, Biomaterials 2006, 27, 5490.
[152] P. Shokrollahi, H. Mirzadeh, O. A. Scherman, W. T. Huck, Journal of biomedical materials research. Part A 2010, 95, 209.
[153] P. Y. Dankers, M. J. van Luyn, A. Huizinga-van der Vlag, G. M. van Gemert, A. H. Petersen, E. W. Meijer, H. M. Janssen, A. W. Bosman, E. R. Popa, Biomaterials 2012, 33, 5144.
[154] P. Y. Dankers, T. M. Hermans, T. W. Baughman, Y. Kamikawa, R. E. Kieltyka, M. M. Bastings, H. M. Janssen, N. A. Sommerdijk, A. Larsen, M. J. van Luyn, A. W. Bosman, E. R. Popa, G. Fytas, E. W. Meijer, Advanced materials 2012, 24, 2703.
[155] K. Nagahama, Y. Ueda, T. Ouchi, Y. Ohya, Biomacromolecules 2009, 10, 1789.
[156] C. Wischke, A. T. Neffe, S. Steuer, A. Lendlein, Journal of Controlled Release 2009, 138, 243; P. Blasi, S. S. D'Souza, F. Selmin, P. P. DeLuca, Journal of Controlled Release 2005, 108, 1.
[157] Y. K. Feng, S. F. Zhang, H. Y. Wang, H. Y. Zhao, J. Lu, J. T. Guo, M. Behl, A. Lendlein, Journal of Controlled Release 2011, 152, E21.
[158] D. J. Maitland, M. F. Metzger, D. Schumann, A. Lee, T. S. Wilson, Laser Surg Med 2002, 30, 1.
[159] A. L. a. R. Langer, Science 2002, 296, 1673.
[160] J. M. Hampikian, B. C. Heaton, F. C. Tong, Z. Q. Zhang, C. P. Wong, Mat Sci Eng C-Bio S 2006, 26, 1373.
[161] W. Small, P. Singhal, T. S. Wilson, D. J. Maitland, J Mater Chem 2010, 20, 3356; A. Lendlein, J. Zotzmann, Y. K. Feng, A. Alteheld, S. Kelch, Biomacromolecules 2009, 10, 975; T. S. Wilson, J. P. Bearinger, J. L. Herberg, J. E. Marion, W. J. Wright, C. L. Evans, D. J. Maitland, J Appl Polym Sci 2007, 106, 540.
[162] M. C. Serrano, G. A. Ameer, Macromolecular bioscience 2012, 12, 1156; A. Metcalfe, A. C. Desfaits, I. Salazkin, L. Yahia, W. M. Sokolowski, J. Raymond, Biomaterials 2003, 24, 491.
[163] T. F. Wu, M. Frydrych, K. O'Kelly, B. Q. Chen, Biomacromolecules 2014, 15, 2663.
[164] J. K. Chen, C. J. Chang, Materials 2014, 7, 805; K. Stubenrauch, I. Voets, G. Fritz-Popovski, G. Trimmel, J Polym Sci Pol Chem 2009, 47, 1178; D. Dupin, J. Rosselgong, S. P. Armes, A. F. Routh, Langmuir 2007, 23, 4035; J. W. Lee, D. S. Lee, S. W. Kim, Macromol Res 2003, 11, 189; C. A. Schoener, H. N. Hutson, N. A. Peppas, Polym Int 2012, 61, 874.
[165] H. B. Lu, W. M. Huang, F. Liang, K. Yu, Materials 2013, 6, 3742.
[166] J. W. Cho, J. W. Kim, Y. C. Jung, N. S. Goo, Macromol Rapid Comm 2005, 26, 412; I. S. Gunes, G. A. Jimenez, S. C. Jana, Carbon 2009, 47, 981; Q. H. Meng, J. L. Hu, Compos Part a-Appl S 2009, 40, 1661; J. S. Leng, X. Lan, Y. J. Liu, S. Y. Du, Prog Mater Sci 2011, 56, 1077; N. S. Goo, I.

H. Paik, K. J. Yoon, Smart Materials & Structures 2007, 16, N23; I. H. Paik, N. S. Goo, Y. C. Jung, J. W. Cho, Smart Materials & Structures 2006, 15, 1476.
[167] S. A. Madbouly, A. Lendlein, Adv Polym Sci 2010, 226, 41; C. L. Ondeck, A. H. Habib, P. Ohodnicki, K. Miller, C. A. Sawyer, P. Chaudhary, M. E. McHenry, J Appl Phys 2009, 105; P. R. Buckley, G. H. McKinley, T. S. Wilson, W. Small, W. J. Benett, J. P. Bearinger, M. W. McElfresh, D. J. Maitland, Ieee T Bio-Med Eng 2006, 53, 2075; C. M. Yakacki, N. S. Satarkar, K. Gall, R. Likos, J. Z. Hilt, J Appl Polym Sci 2009, 112, 3166.
[168] H. Zhang, H. T. Wang, W. Zhong, Q. G. Du, Polymer 2009, 50, 1596.
[169] G. M. Baer, W. Small, T. S. Wilson, W. J. Benett, D. L. Matthews, J. Hartman, D. J. Maitland, Biomed Eng Online 2007, 6.
[170] I. Bellin, S. Kelch, R. Langer, A. Lendlein, Proceedings of the National Academy of Sciences of the United States of America 2006, 103, 18043.
[171] L. A. Xue, S. Y. Dai, Z. Li, Biomaterials 2010, 31, 8132.
[172] F. Migneco, Y. C. Huang, R. K. Birla, S. J. Hollister, Biomaterials 2009, 30, 6479.
[173] S. Neuss, I. Blomenkamp, R. Stainforth, D. Boltersdorf, M. Jansen, N. Butz, A. Perez-Bouza, R. Knuchel, Biomaterials 2009, 30, 1697.
[174] Y. J. Liu, H. Y. Du, L. W. Liu, J. S. Leng, Smart Mater Struct 2014, 23.
[175] W. Sokolowski, Open Medical Devices Journal 2010, 2, 20.
[176] W. M. Huang, B. ang, Y. Q. Fu, CRC Press 2011.
[177] *Smart Polymers and their Applications*, Elsevier, 2014.
[178] J. L. Hu, S. J. Chen, J Mater Chem 2010, 20, 3346; L. Sun, W. M. Huang, Z. Ding, Y. Zhao, C. C. Wang, H. Purnawali, C. Tang, Mater Design 2012, 33, 577.
[179] X. F. Luo, P. T. Mather, Adv Funct Mater 2010, 20, 2649; J. Cui, K. Kratz, M. Heuchel, B. Hiebl, A. Lendlein, Polym Advan Technol 2011, 22, 180.
[180] M. R. Aguilar, J. San Roman, Woodhead Publ Mater 2014, 1.
[181] Q. Zhao, M. Behl, A. Lendlein, Soft Matter 2013, 9, 1744; Y. W. Luo, Y. L. Guo, X. Gao, B. G. Li, T. Xie, Advanced materials 2013, 25, 743.
[182] M. Irie, D. Kunwatchakun, Macromolecules 1986, 19, 2476.
[183] D. Iqbal, M. H. Samiullah, Materials 2013, 6, 116.
[184] M. Behl, A. Lendlein, Mater Today 2007, 10, 20.
[185] A. T. Neffe, B. D. Hanh, S. Steuer, A. Lendlein, Advanced materials 2009, 21, 3394; A. T. Neffe, B. D. Hanh, S. Steuer, C. Wischke, A. Lendlein, Mater Res Soc Symp P 2009, 1190, 105; C. Wischke, A. T. Neffe, S. Steuer, A. Lendlein, European Journal of Pharmaceutical Sciences 2010, 41, 136; C. Wischke, S. Steuer, A. Lendlein, Multifunctional Polymer-Based Materials 2012, 1403, 207.
[186] J. N. Rodriguez, Y. J. Yu, M. W. Miller, T. S. Wilson, J. Hartman, F. J. Clubb, B. Gentry, D. J. Maitland, Annals of biomedical engineering 2012, 40, 883.
[187] A. Lendlein, R. Langer, Science 2002, 296, 1673.
[188] G. M. Baer, W. t. Small, T. S. Wilson, W. J. Benett, D. L. Matthews, J. Hartman, D. J. Maitland, Biomed Eng Online 2007, 6, 43.
[189] M. C. Chen, H. W. Tsai, Y. Chang, W. Y. Lai, F. L. Mi, C. T. Liu, H. S. Wong, H. W. Sung, Biomacromolecules 2007, 8, 2774.
[190] L. Xue, S. Dai, Z. Li, Biomaterials 2010, 31, 8132.
[191] N. Roy, B. Bruchmann, J.-M. Lehn, Chem Soc Rev 2015, 44, 3786; R. J. Dong, Y. F. Zhou, X. H. Huang, X. Y. Zhu, Y. F. Lu, J. Shen, Advanced materials 2015, 27, 498; C. Heinzmann, C. Weder, L. M. de Espinos, Chemical Sociery Reviews 2015.
[192] H. N. Chia, B. M. Wu, J Biol Eng 2015, 9.
[193] P. Roach, D. Eglin, K. Rohde, C. C. Perry, Journal of Materials Science-Materials in Medicine 2007, 18, 1263.
[194] S. G. Zhang, F. Gelain, X. J. Zhao, Seminars in Cancer Biology 2005, 15, 413.
[195] A. Selimis, V. Mironov, M. Farsari, Microelectronic Engineering 2015, 132, 83.

[196] E. Stratakis, A. Ranella, M. Farsari, C. Fotakis, Progress in Quantum Electronics 2009, 33, 127.
[197] A. Ovsianikov, V. Mironov, J. Stampfl, R. Liska, Expert Rev. Med. Dev. 2012, 9, 613.
[198] J. A. Barron, B. R. Ringeisen, H. S. Kim, B. J. Spargo, D. B. Chrisey, Thin Solid Films 2004, 453, 383; F. Guillemot, B. Guillotin, A. Fontaine, M. Ali, S. Catros, V. Keriquel, J. C. Fricain, M. Remy, R. Bareille, J. Amedee-Vilamitjana, MRS Bull. 2011, 36, 1015; L. Koch, M. Gruene, C. Unger, B. Chichkov, Curr. Pharm. Biotechnol. 2013, 14, 91; V. Dinca, A. Ranella, M. Farsari, D. Kafetzopoulos, M. Dinescu, A. Popescu, C. Fotakis, Biomed. Microdevices 2008, 10, 719; M. Duocastella, A. Patrascioiu, J. M. Fernandez-Pradas, J. L. Morenza, P. Serra, Opt. Express 2010, 18, 21815; C. Boutopoulos, M. Chatzipetrou, A. G. Papathanasiou, I. Zergioti, Laser Phys. Lett. 2014, 11, 8.
[199] B. R. Ringeisen, H. Kim, J. A. Barron, D. B. Krizman, D. B. Chrisey, S. Jackman, R. Y. C. Auyeung, B. J. Spargo, Tissue Engineering 2004, 10, 483; B. Hopp, T. Smausz, G. Szabo, L. Kolozsvari, D. Kafetzopoulos, C. Fotakis, A. Nogradi, Optical Engineering 2012, 51, 10.1117/1.oe.51.1.014302.
[200] A. Palla-Papavlu, I. Paraico, J. Shaw-Stewart, V. Dinca, T. Savopol, E. Kovacs, T. Lippert, A. Wokaun, M. Dinescu, Appl. Phys. A-Mater. Sci. Process. 2011, 102, 651.
[201] F. Guillemot, B. Guillotin, A. Fontaine, M. Ali, S. Catros, V. Kériquel, J.-C. Fricain, M. Rémy, R. Bareille, J. Amédée-Vilamitjana, MRS Bull. 2011, 36, 1015.
[202] M. Gruene, C. Unger, L. Koch, A. Deiwick, B. Chichkov, Biomed Eng Online 2011, 10, 11; J. Y. Yan, Y. Huang, D. B. Chrisey, Biofabrication 2013, 5, 8.
[203] B. R. Ringeisen, P. K. Wu, H. Kim, A. Pique, R. Y. C. Auyeung, H. D. Young, D. B. Chrisey, D. B. Krizman, Biotechnology Progress 2002, 18, 1126.
[204] B. Guillotin, A. Souquet, S. Catros, M. Duocastella, B. Pippenger, S. Bellance, R. Bareille, M. Remy, L. Bordenave, J. Amedee, F. Guillemot, Biomaterials 2010, 31, 7250.
[205] P. K. Wu, B. R. Ringeisen, Biofabrication 2010, 2, 8.
[206] R. Gaebel, N. Ma, J. Liu, J. Guan, L. Koch, C. Klopsch, M. Gruene, A. Toelk, W. Wang, P. Mark, F. Wang, B. Chichkov, W. Li, G. Steinhoff, Biomaterials 2011, 32, 9218.
[207] S. Michael, H. Sorg, C.-T. Peck, L. Koch, A. Deiwick, B. Chichkov, P. M. Vogt, K. Reimers, PloS one 2013, 8, e57741.
[208] E. Berry, A. Marsden, K. W. Dalgarno, D. Kessel, D. J. A. Scott, Proc. Inst. Mech. Eng. Part H-J. Eng. Med. 2002, 216, 211.
[209] M. Y. Lee, W. W. Tsai, H. J. Chen, J. P. Chen, C. H. Chen, W. L. Yeh, J. An, Bio-Med. Mater. Eng. 2013, 23, 533; J. M. Williams, A. Adewunmi, R. M. Schek, C. L. Flanagan, P. H. Krebsbach, S. E. Feinberg, S. J. Hollister, S. Das, Biomaterials 2005, 26, 4817; A. Mazzoli, Med. Biol. Eng. Comput. 2013, 51, 245.
[210] S. Lohfeld, S. Cahill, V. Barron, P. McHugh, L. Durselen, L. Kreja, C. Bausewein, A. Ignatius, Acta Biomater 2012, 8, 3446.
[211] H. Huang, S. Oizumi, N. Kojima, T. Niino, Y. Sakai, Biomaterials 2007, 28, 3815.
[212] W. Y. Yeong, N. Sudarmadji, H. Y. Yu, C. K. Chua, K. F. Leong, S. S. Venkatraman, Y. C. F. Boey, L. P. Tan, Acta Biomater 2010, 6, 2028.
[213] T. Matsuda, M. Mizutani, Macromolecules 2000, 33, 791.
[214] F. P. W. Melchels, M. A. N. Domingos, T. J. Klein, J. Malda, P. J. Bartolo, D. W. Hutmacher, Prog Polym Sci 2012, 37, 1079.
[215] K. C. Hribar, P. Soman, J. Warner, P. Chung, S. C. Chen, Lab Chip 2014, 14, 268; F. P. W. Melchels, J. Feijen, D. W. Grijpma, Biomaterials 2010, 31, 6121; S. J. Hollister, Nature materials 2005, 4, 518.
[216] J. P. Fisher, J. W. M. Vehof, D. Dean, J. van der Waerden, T. A. Holland, A. G. Mikos, J. A. Jansen, J. Biomed. Mater. Res. 2002, 59, 547; J. W. Lee, K. S. Kang, S. H. Lee, J. Y. Kim, B. K. Lee, D. W. Cho, Biomaterials 2011, 32, 744.
[217] F. P. W. Melchels, J. Feijen, D. W. Grijpma, Biomaterials 2009, 30, 3801.

[218] C. Wei, L. Cai, B. Sonawane, S. F. Wang, J. Y. Dong, Biofabrication 2012, 4, 12.
[219] S. Khetan, J. A. Burdick, Soft Matter 2011, 7, 830; K. Arcaute, B. K. Mann, R. B. Wicker, Annals of biomedical engineering 2006, 34, 1429.
[220] L. A. Hockaday, K. H. Kang, N. W. Colangelo, P. Y. C. Cheung, B. Duan, E. Malone, J. Wu, L. N. Girardi, L. J. Bonassar, H. Lipson, C. C. Chu, J. T. Butcher, Biofabrication 2012, 4, 12.
[221] S. Schuller-Ravoo, E. Zant, J. Feijen, D. W. Grijpma, Adv Healthc Mater 2014, 3, 2004.
[222] Y. H. Joo, J. H. Park, D. W. Cho, D. I. Sun, Tissue Eng. Regen. Med. 2013, 10, 65.
[223] M. Malinauskas, M. Farsari, A. Piskarskas, S. Juodkazis, Physics Reports 2013, 533, 1.
[224] M. Malinauskas, P. Danilevicius, D. Baltriukiene, M. Rutkauskas, A. Zukauskas, Z. Kairyte, G. Bickauskaite, V. Purlys, D. Paipulas, V. Bukelskiene, R. Gadonas, Lith. J. Phys. 2010, 50, 75; T. Honegger, T. Elmberg, K. Berton, D. Peyrade, Microel. Engin. 2011, 88, 2725; A. Ovsianikov, M. Malinauskas, S. Schlie, B. Chichkov, S. Gittard, R. Narayan, M. Lobler, K. Sternberg, K. P. Schmitz, A. Haverich, Acta Biomater. 2011, 7, 967; H. Zhang, F. Landmann, H. Zahreddine, D. Rodriguez, M. Koch, M. Labouesse, Nature 2011, 471, 99; W. D. Zhang, P. Soman, K. Meggs, X. Qu, S. C. Chen, Adv. Funct. Mater. 2013, 23, 3226; C. N. LaFratta, J. T. Fourkas, T. Baldacchini, R. A. Farrer, Angew Chem Int Edit 2007, 46, 6238; M. Farsari, G. Filippidis, K. Sambani, T. S. Drakakis, C. Fotakis, Journal of Photochemistry and Photobiology a-Chemistry 2006, 181, 132; S. Juodkazis, V. Mizeikis, H. Misawa, J. Appl. Phys. 2009, 106, 051101; V. Dinca, E. Kasotakis, J. Catherine, A. Mourka, A. Ranella, A. Ovsianikov, B. N. Chichkov, M. Farsari, A. Mitraki, C. Fotakis, Nano Letters 2008, 8, 538; M. T. Raimondi, S. M. Eaton, M. Lagana, V. Aprile, M. M. Nava, G. Cerullo, R. Osellame, Acta Biomater. 2013, 9, 4579; K. Terzaki, E. Kalloudi, E. Mossou, E. P. Mitchell, V. T. Forsyth, E. Rosseeva, P. Simon, M. Vamvakaki, M. Chatzinikolaidou, A. Mitraki, M. Farsari, Biofabrication 2013, 5, 045002; K. Terzaki, M. Kissamitaki, A. Skarmoutsou, C. Fotakis, C. A. Charitidis, M. Farsari, M. Vamvakaki, M. Chatzinikolaidou, J. Biomed. Mater. Res. A 2013, 101A, 2283; M. Chatzinikolaidou, S. Rekstyte, P. Danilevicius, C. Pontikoglou, H. Papadaki, M. Farsari, M. Vamvakaki, Materials Science and Engineering: C 2015, 48, 301.
[225] J. D. Pitts, P. J. Campagnola, G. A. Epling, S. L. Goodman, Macromol. 2000, 33, 1514; J. D. Pitts, A. R. Howell, R. Taboada, I. Banerjee, J. Wang, S. L. Goodman, P. J. Campagnola, Photochem. Photobiol. 2002, 76, 135; S. Basu, P. J. Campagnola, J. Biomed. Mater. Res. A 2004, 71A, 359; S. Basu, P. J. Campagnola, Biomacromol. 2004, 5, 572; S. Basu, L. P. Cunningham, G. D. Pins, K. A. Bush, R. Taboada, A. R. Howell, J. Wang, P. J. Campagnola, Biomacromol. 2005, 6, 1465; L. P. Cunningham, M. P. Veilleux, P. J. Campagnola, Opt. Exp. 2006, 14, 8613; P. J. Su, Q. A. Tran, J. J. Fong, K. W. Eliceiri, B. M. Ogle, P. J. Campagnola, Biomacromol. 2012, 13, 2917.
[226] A. Ovsianikov, A. Deiwick, S. Van Vlierberghe, P. Dubruel, L. Möller, G. Dräger, B. Chichkov, Biomacromolecules 2011, 12, 851; X. H. Qin, J. Torgersen, R. Saf, S. Muhleder, N. Pucher, S. C. Ligon, W. Holnthoner, H. Redl, A. Ovsianikov, J. Stampfl, R. Liska, J. Polym. Sci. Pol. Chem. 2013, 51, 4799.
[227] O. Kufelt, A. El-Tamer, C. Sehring, S. Schlie-Wolter, B. N. Chichkov, Biomacromol. 2014, 15, 650.
[228] F. Claeyssens, E. A. Hasan, A. Gaidukeviciute, D. S. Achilleos, A. Ranella, C. Reinhardt, A. Ovsianikov, S. Xiao, C. Fotakis, M. Vamvakaki, B. N. Chichkov, M. Farsari, Langmuir 2009, 25, 3219.
[229] V. Melissinaki, A. A. Gill, I. Ortega, M. Vamvakaki, A. Ranella, J. W. Haycock, C. Fotakis, M. Farsari, F. Claeyssens, Biofabrication 2011, 3, 045005.
[230] P. Danilevicius, L. Georgiadi, C. J. Pateman, F. Claeyssens, M. Chatzinikolaidou, M. Farsari, Applied Surface Science 2015, DOI: 10.1016/j.apsusc.2014.06.012.
[231] S. K. Seidlits, C. E. Schmidt, J. B. Shear, Adv Funct Mater 2009, 19, 3543.
[232] J. Torgersen, A. Ovsianikov, V. Mironov, N. Pucher, X. H. Qin, Z. Q. Li, K. Cicha, T. Machacek, R. Liska, V. Jantsch, J. Stampfl, J. Biomed. Opt. 2012, 17.
[233] H. Tran, K. L. Killops, L. M. Campos, Soft Matter 2013, 9, 6578.

[234] G. Möllenstedt, R. Speidel, Physik Journal 1960, 16, 192.
[235] C. P. Tan, B. R. Cipriany, D. M. Lin, H. G. Craighead, Nano Letters 2010, 10, 719; A. Pallandre, B. De Meersman, F. Blondeau, B. Nysten, A. M. Jonas, J Am Chem Soc 2005, 127, 4320.
[236] M. Palma, J. J. Abramson, A. A. Gorodetsky, E. Penzo, R. L. Gonzalez, M. P. Sheetz, C. Nuckolls, J. Hone, S. J. Wind, J Am Chem Soc 2011, 133, 7656.
[237] C. Subramani, S. Dickert, Y. C. Yeh, M. T. Tuominen, V. M. Rotello, Langmuir 2011, 27, 1543.
[238] I. Tonazzini, S. Meucci, P. Faraci, F. Beltram, M. Cecchini, Biomaterials 2013, 34, 6027.
[239] M. J. Biggs, R. G. Richards, N. Gadegaard, C. D. Wilkinson, M. J. Dalby, Journal of orthopaedic research : official publication of the Orthopaedic Research Society 2007, 25, 273.
[240] R. J. McMurray, N. Gadegaard, P. M. Tsimbouri, K. V. Burgess, L. E. McNamara, R. Tare, K. Murawski, E. Kingham, R. O. Oreffo, M. J. Dalby, Nature materials 2011, 10, 637.
[241] O. Cherniavskaya, C. J. Chen, E. Heller, E. Sun, J. Provezano, L. Kam, J. Hone, M. P. Sheetz, S. J. Wind, J Vac Sci Technol B 2005, 23, 2972.
[242] M. A. Brenckle, H. Tao, S. Kim, M. Paquette, D. L. Kaplan, F. G. Omenetto, Advanced materials 2013, 25, 2409.
[243] L. C. Glangchai, M. Caldorera-Moore, L. Shi, K. Roy, Journal of Controlled Release 2008, 125, 263.
[244] A. Azeem, A. English, P. Kumar, A. Satyam, M. Biggs, E. Jones, B. Tripathi, N. Basu, J. Henkel, C. Vaquette, N. Rooney, G. Riley, A. O'Riordan, G. Cross, S. Ivanovski, D. Hutmacher, A. Pandit, D. I. Zeugolis, Nanomedicine-Uk 2015, 10, 693.
[245] S. Watari, K. Hayashi, J. A. Wood, P. Russell, P. F. Nealey, C. J. Murphy, D. C. Genetos, Biomaterials 2012, 33, 128.
[246] K. J. Jeon, S. H. Park, J. W. Shin, Y. G. Kang, J. S. Hyun, M. J. Oh, S. Y. Kim, J. W. Shin, Journal of bioscience and bioengineering 2014, 117, 242.
[247] P. Y. Wang, W. T. Li, J. Yu, W. B. Tsai, Journal of materials science. Materials in medicine 2012, 23, 3015.
[248] B. K. K. Teo, S. T. Wong, C. K. Lim, T. Y. S. Kung, C. H. Yap, Y. Ramagopal, L. H. Romer, E. K. F. Yim, ACS nano 2013, 7, 4785.
[249] M. Schvartzman, M. Palma, J. Sable, J. Abramson, X. A. Hu, M. P. Sheetz, S. J. Wind, Nano Letters 2011, 11, 1306.
[250] F. Pati, J. Jang, D. H. Ha, S. Won Kim, J. W. Rhie, J. H. Shim, D. H. Kim, D. W. Cho, Nat Commun 2014, 5, 3935.
[251] A. V. Bryksin, A. C. Brown, M. M. Baksh, M. G. Finn, T. H. Barker, Acta Biomater 2014, 10, 1761.

Fig 1
Click here to download Production Data: Fig 1.jpg

Fig 2
Click here to download Production Data: Fig 2.jpg

Fig 3
Click here to download Production Data: Fig 3.jpg

Fig 4
Click here to download Production Data: Fig 4.jpg

Fig 5
Click here to download Production Data: Fig 5.jpg

Fig 6
Click here to download Production Data: Fig 6.jpg

Fig 7
Click here to download Production Data: Fig 7.jpg

Fig 8
Click here to download Production Data: Fig 8.jpg

Fig 9
Click here to download Production Data: Fig 9.jpg

Fig 10
Click here to download Production Data: Fig 10.jpg

Fig 11
Click here to download Production Data: Fig 11.jpg